S/N 09/647,054 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Peter Joseph Cassidy, et al.	Examiner: Christopher M. Gross
Serial No.:	09/647,054	Group Art Unit: 1639
Filed:	March 24, 1998	Docket No.: 707.025US1
Title:	PEPTIDE TURN MIMETICS	

DECLARATION UNDER 37 C.F.R. §1.132

I, Peter Joseph Cassidy, declare and say as follows:

1. I, Peter Joseph Cassidy, received my bachelor's and doctorate degrees at the University of Queensland, Brisbane, Australia.

2. I am a named co-inventor of the subject matter claimed in the above-identified patent application and have reviewed the summary provided by the attorneys for Mimetica of the interview that was conducted at the USPTO on 13 November 2008 between patent examiner Christopher Gross, supervising examiner Mark Shibuya, Mark Blaskovich of Mimetica Pty Ltd and Geoffrey Cooper and Gary Speier of Schwegmann, Lundberg and Woessner. I hereby make this Declaration in support of the patentability of the claims of the application.

3. I understand that one of the main points of the discussion at the interview was in relation to the fact that the Examiner has rejected claims 113, 119, 120, 121, 124, 126, 134, 135, 137, 138, and 140 on the basis of 35 U.S.C. §102(b) as being anticipated by Ma et al., 1995, Protein Peptide Letters, 2:347-350. I understand that there was extensive discussion of the data that would be required in support of the applicant's position that the Ma disclosure does not anticipate or render obvious the claims of the present application on the basis that following the procedure in Ma does not produce the compound alleged by Ma but rather an isomer of this compound.

4. As a result of the interview with the examiner the applicant initiated an experimental program aimed at providing the data required to satisfy the examiner that the Ma procedure did not in fact produce the compounds alleged, and specifically did not produce a compound within the scope of the claims of the current application.

5. I attach as Appendix 1 a report prepared based on the experiments carried out. In order to avoid any possible role of trace impurities having an effect on the cyclisation studies carried out in the report the cyclisation precursor 10 was prepared by the same method as described in Ma.

6. The first steps in this process were as shown in scheme 1 on page 3 of the report and involved production of compound(s) of formula 7 which were produced as a mixture of epimers. The ^{1}H NMR and ^{13}C NMR for the compound(s) of formula 7 are shown in appendix 2.

7. This mixture of epimers was then converted to compounds of formula 8. The ^{1}H NMR, ^{13}C NMR and mass spectral data of the isomers of formula 8 is shown in appendix 3.

8. This mixture of free amines 8 was then converted to the protected forms 9 under the conditions taught by Ma. The ^{1}H NMR and ^{13}C NMR for the compound(s) of formula 9 are shown in appendix 4.

9. This mixture of the protected amines (9) were then reacted to form the cyclisation precursor 10 under the conditions taught by Ma. The ^{1}H NMR and ^{13}C NMR for the compound(s) of formula 10 are shown in appendix 5.

10. Once the cyclisation precursor 10 was in hand and prior to conducting the factorial Mitsunobu experiments under a variety of conditions it was decided to produce

the original diazepane target (compound 2) as allegedly produced by Ma using the applicants own chemistry as outlined in scheme 2 (page 3 of the report). An advanced intermediate in this synthesis was compound 14 and the data obtained for this compound are shown in appendix 6.

11. The compound of formula 14 was then protected on nitrogen to produce compound 2 and the data obtained for this compound are shown in appendix 7.

12. With the authentic sample of compound 2 in hand the isomers of 10 were subjected to the Mitsunobu reaction as detailed in Ma. The results were compounds 3a and 3b. The data obtained for compound 3a (formed from cyclisation of compound 10a) is shown in appendix 8. The data obtained for compound 3b (formed from cyclisation of compound 10b) is shown in appendix 9.

13. As can be seen cyclisation of compounds 10 did not produce the compound 2 as made by the Mimetica chemistry. Analysis of the cyclisation products 3 clearly demonstrated that the products retained the Alanine nitrogen proton (as discussed on page 4 of the report) indicating that the cyclisation product 2 (which does not have this moiety) could not be the structure.

14. In order to determine whether the Mitsunobu conditions affected the cyclisation reaction a number of different conditions were trialled as shown in page 7 and 8 of the report. There was no appreciable difference in the reaction products obtained irrespective of the reaction conditions used. The mass spectral analysis of the products produced in the factorial experiments is shown in appendix 10, while the HPLC spectra are shown in appendix 11 and appendix 12.

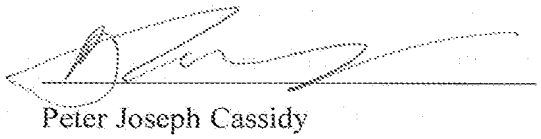
15. As discussed in the report the HPLC data obtained was particularly significant. As discussed in the report the authentic cyclised compound 2 (which Ma alleged was made in the reactions) had a retention time of approximately 8.71 minutes

whereas the two aziridine products had retention times of 6.87 and 6.93 minutes respectively. In addition even when a mixture of authentic product (2) and a crude reaction mixture from a cyclisation reaction is injected as a co-injection there is no significant change in retention time for the authentic compound (2). The HPLC traces from the factorial experiments indicate that irrespective of the reaction conditions there was no observable quantity of the compound (2) produced.

16. In my professional judgement, these data prove that under the reaction conditions disclosed by Ma, and under a range of reaction conditions that are within the due experimental exploration of a person of ordinary skill in the art, the products obtained do not contain in any appreciable amount any of the chemical structure asserted by Ma to be formed.

17. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application or any patent issuing therefrom.

24/10/2008
Date



Peter Joseph Cassidy

APPENDIX 1

Report to the US Patent Office

mimetica

MIMETICA PTY LIMITED

REPORT TO THE US PATENT OFFICE

Supporting Data for US patent application 09/647054
(Peptide Turn Mimetics).

Factorial experiments to refute Ma et al.

OCTOBER 22, 2008

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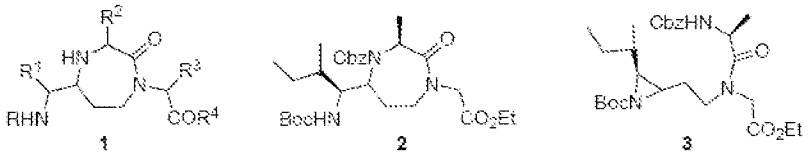
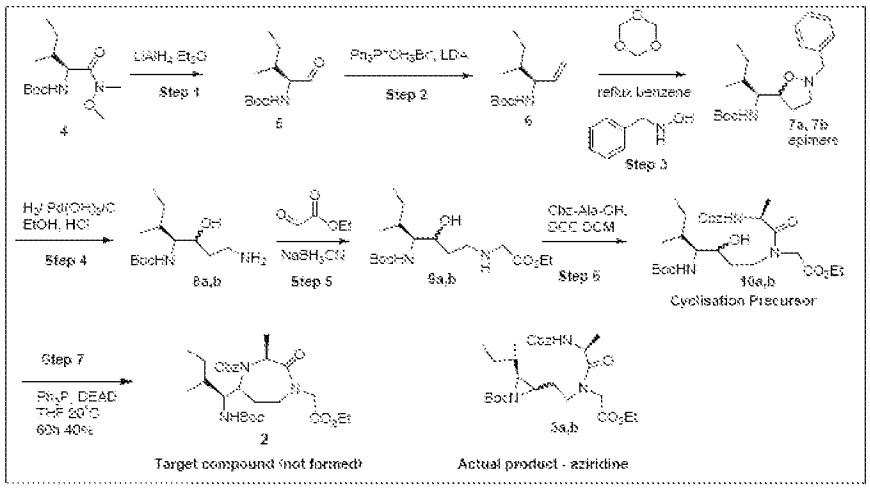
Factorial Mitsunobu Experiments to Refute Ma et al.

Background

US patent application 09/647054 (Mimetica Pty Ltd) ("Peptide Turn Mimetics", following from PCT/AU1999/0007(WO/1999/048913)) claims various peptide mimetic compounds and methods for their synthesis. The compounds claimed include 1,4-diazepanones of general structure **1**.



A publication before to the priority date of the application (*Ma et al.* Prot Pept Lett 1995 p347-350) claims to provide a synthesis of a diazepane **2**, a structure within the claims of the application. Evidence was provided in the original patent application that the work described in the Ma publication ("Ma procedure", Scheme 1) does not represent relevant prior art for the Peptide Turn Mimetics application because the Ma procedure forms the isomeric compound **3** (not claimed in the application) and not the diazepane **2**. Compounds **2** and **3** have the same molecular weight but can be easily differentiated by NMR spectroscopy.



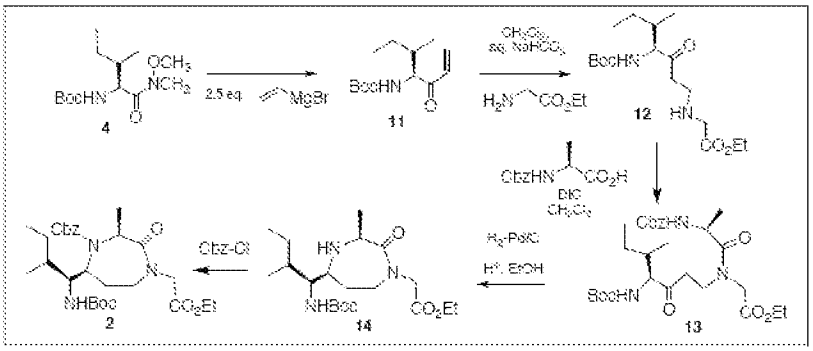
Scheme 1. Ma procedure (*Ma et al.* Prot Pept Lett 1995 p347-350)

The US patent office has requested further reactions be conducted to confirm the assertion that the Ma procedure does not produce compound **2**. Specifically requested were:

(1) Preparation of the cyclisation precursor **10** by the same method as described in the Ma procedure to rule out the role of trace impurities in directing the reaction outcome;
(2) Completion of factorial cyclisation experiments to demonstrate that reasonable variations in the reaction conditions, particularly order of addition of the reagents, do not alter the reaction outcome.

Summary of Results

The requested experiments have been completed and have confirmed the original finding, i.e. that the cyclisation product is aziridine **3** and not the claimed **2**. In addition and for greater certainty the original diazepane target compound **2** from the Ma publication has been prepared using Mimetica's chemistry (as described in Scheme 2). This sample has been used to provided reference NMR and mass spectra and chromatographs to compare with the products of the factorial reactions. No trace of this reference material or any other stereoisomer of **2** was detected in any of the factorial Mitsunobu reaction products.



Scheme 2. Preparation of authentic Ma target compound using the Mimetica procedure.

Discussion

The ^{1}H and ^{13}C NMR spectra of intermediates and the final products are listed in the experimental section below. Copies of the spectra including COSY and TOCSY spectra of key compounds are included as attachments. The Ma publication provided limited characterisation data – the only relevant spectrum being unassigned ^{1}H NMR data for the mixed epimers of the product material. No copy of the spectrum was available.

(1) Preparation of compound 10 by the procedure of Ma et al.

The isomers of the alcohol cyclisation pre-cursor **10** were prepared in accordance with the procedure of Ma et al (Scheme 1). The material prepared was compared to material previously prepared by reduction of compound **13**: as expected the products were the same according to NMR spectra except the ratio of epimers was different – about 6:4 for the Ma procedure against 1:7 for the borohydride reduction of **13**. Sufficient separation of the isomers was achieved by flash chromatography to enable separate analysis.

The Alanine residue spin system with NH coupling to Hα then to $CH_3\beta$ is easily identified in the ^1H NMR spectra of **10**, the NH falling at 5.65 and 5.46 ppm (two signals due to secondary amide cis/trans conformers) coupled to the Hα at 4.79 and 4.44 and then to the $CH_3\beta$ at 1.38 and 1.33 ppm. On cyclisation if product **2** is formed the Ala spin system will change due to loss of the NH in the cyclisation.

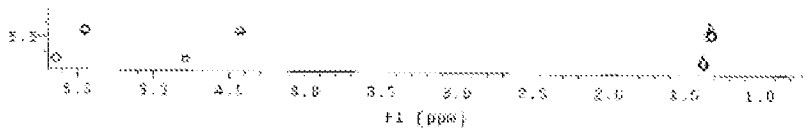


Figure 1. Excerpt from the TOCSY spectrum of compound **10a** (PCM425, first eluting epimer) illustrating the Ala spin system of the two amide conformers. The left hand signals are from the NH and the spectrum shows these are coupled to the Hα and Hβ positions.

(2) Mitsunobu Reaction Products

Mitsunobu reaction conditions were applied to **10** and as previously reported (in the patent application) a dehydration product was formed from each of the epimers. The products formed did not vary according to the reaction conditions or to the ratio of epimers in the starting material. The products were purified by flash chromatography and analysed by NMR and mass spectrometry. The ^1H NMR spectra of the products clearly show the retention of the Ala NH (at 5.68 and 5.66 ppm) – this is impossible if the product is **2** as suggested by Ma et al. The data is summarised in Table 1 for one of the epimers.

	Ala NH	Ala Hα	Ala Hβ
Compound 10a	5.65, 5.46	4.79, 4.44	1.38, 1.33
Mitsunobu Product	5.68, 5.66	4.73, 4.48	1.43, 1.32

Table 1. Chemical shifts of the Alanine spin system in starting alcohol **10** and the corresponding Mitsunobu cyclisation product.

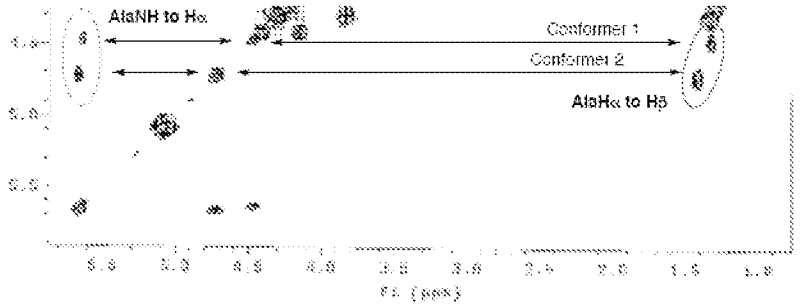


Figure 2. Excerpt from the COSY spectrum of Mitsunobu cyclisation product (**3a**, PCM432) formed from compound **10a** containing the Ala spin system and showing retention of the NH signal.

In contrast to the lack of change at the Ala NH position, the Ile NH position disappeared and there were significant changes to the Ile system including movement of the IleHα signal from 3.18 to 2.18/2.13 ppm. These changes indicate a cyclisation involving the IleNH with

the product being the azidirine **3**. Further information on the shift changes on formation of compounds **3** and comparison to literature aziridine NMR data is included in the Experimental Section data for **3** (page 13).

(3) Preparation of Authentic Turn Mimetic 2.

To further assist in confirming these findings we prepared compound **2** using the method described in Scheme 2. Reductive amination-cyclization to form **14** from **13** gave a single isomer. This compound has no amide rotamers and hence has more easily analysed NMR spectra. Reaction to form **2** is complicated by the high level of steric hindrance around the ring amine – it was accomplished using neat Cbz-Cl in aqueous $NaHCO_3$ at 40°C. Compound **2** displays multiple conformers due to secondary amide rotamers and also restricted rotation at the Ile group due to crowding. Variable temperature NMR was used to demonstrate that the conformers were all due to a single compound. The authentic product has clearly different spectra from either of the Mitsunobu reaction products and also from the limited data reported by Ma et al. (see Appendix 1 for a tabular comparison highlighting the differences).

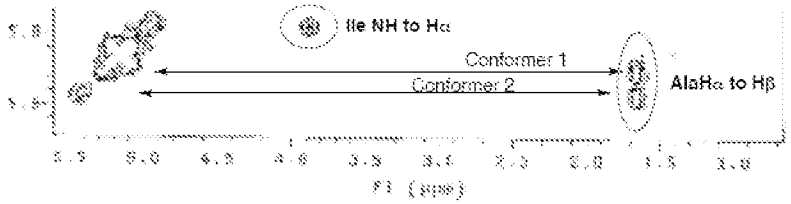


Figure 3. Excerpt from the COSY spectrum of authentic target mimetic **2** (PCM416) containing the Ala spin system showing Hα to Hβ coupling and absence of an NH to Hα coupling for the two conformers. Also illustrated is the Ile NH to Hα cross peak – this is not present in the Mitsunobu products **3** due to the IleN being in an azirindine ring.

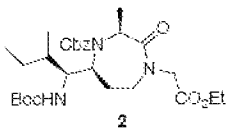
(4) Mass Spectra of Product and Target Compounds

The mass spectra of the products formed under the Mitsunobu cyclisation conditions and also of the authentic mimetic were examined. The fragmentation patterns show significant differences with a number of unique ions enabling clear differentiation of the isomeric compounds. Specifically, on fragmentation both epimers of the aziridine **3** form unique fragmentation products, notably one of mass 273^+ with high relative intensity. The authentic target **2** produces unique ions of mass 406^+ and 277^+ at the same fragmentation energy. The 273^+ ion was detected in all of the factorial cyclisation experiments, however no trace of the 406^+ and 277^+ ions characteristic of the target **2** were detected in any of the Mitsunobu experiments. Mass spectra from all reactions are included in the attachments and a selection are also illustrated in Figure 4 in the Factorial Experiments Section below. The following table lists the spectra and highlights the unique ions.

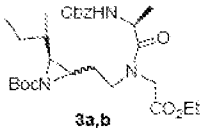
	AZIRIDINE 3A	AZIRIDINE 3B	AUTHENTIC 2	ALCOHOL 10
Parent mass (MH^+)	534	534	534	552
Ion Mass:				
552	13%	38%	-	44%
534	59%	85%	21%	-
496	6%	20%	-	6%
478	100%	84%	24%	-
452	-	-	-	100%
434	44%	67%	100%	13%
406	-	-	9%	-
370	12%	30%	-	-
363	13%	26%	-	-
277	-	-	11%	-
273	30%	100%	-	-

Table 2. Mass spectra of key derivatives at a fragmentation setting EP/DP (entrance potential/ declustering potential) of 12 and 100v. The unique ions are highlighted.

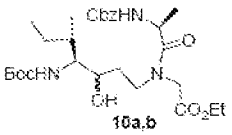
Mass spectra were run on crude products to ensure minor amounts of possible product were not lost during purification.



2



3a,b



10a,b

Factorial Experiments

The following experiments were carried out:

1. Ma conditions of temperature, reagent equivalence, time but using different order of reagent addition, as discussed below (4 experiments);
2. Excess (2 fold) Mitsunobu reagents but otherwise the same conditions (adding DEAD last);
3. Lower temperature (0°C) (adding DEAD last)
4. Higher temperature (40°C) adding DEAD last
5. Using dichloromethane as solvent, DEAD added last
6. Using toluene as solvent, DEAD added last

Order of Addition Experiments

The Mitsunobu reaction used here has three components: DEAD, Ph_3P and alcohol **10**. The most common technique used for the Mitsunobu reaction is to add DEAD to the other components at 0°C. Other regularly used methods have involved the preformation of the betaine by mixing the DEAD and Ph_3P prior to adding the other components (see Organic Reactions, Hughes). Considering the inter-reactivity of the components there are four meaningfully different ways of mixing the components:

i. DEAD added to pre-mixed Ph_3P and alcohol **1**;
ii. Ph_3P added to pre-mixed DEAD and alcohol **1**;
iii. alcohol **1** added to pre-mixed Ph_3P and DEAD where DEAD is initially added dropwise to the Ph_3P solution;
iv. alcohol **1** added to pre-mixed Ph_3P and DEAD where the Ph_3P solution is initially added dropwise to the DEAD solution

REACTION	CONDITIONS	RESULTS AND ANALYSIS
PCM414, mixed isomers ~1:1 ratio	DEAD added last, room temperature, stirred for 72hrs, 20°C	Aziridine formed, purified and NMR run. HPLC and MS analysis
PCM427, first eluting isomer	DEAD added last, room temperature, stirred for 72hrs, 20°C	Aziridine formed, MS analysed
PCM428, mixed isomers ~1:1 ratio	Alcohol added to preformed betaine, DEAD to Ph_3P, 60h, 20°C	Aziridine formed, MS analysed
PCM429, mixed isomers ~1:1 ratio	Double reagents, DEAD last; 60h, 20°C	Aziridine formed, MS analysed
PCM430, mixed isomers ~1:1 ratio	Ph_3P added to alcohol and DEAD, 60h, 20°C	Aziridine formed, MS analysed
PCM431, mixed isomers ~1:1 ratio	DEAD added last at 0°C and allowed to warm to 20°C overnight,	Aziridine formed, MS analysed
PCM432, mixed isomers ~1:1 ratio	Alcohol added to betaine formed from Ph_3P to DEAD	Aziridine formed, MS analysed, purified and NMR
PCM433, mixed isomers ~1:1 ratio	DEAD added last, temperature of 40°C 24h	Aziridine formed, MS analysed
PCM434, mixed isomers ~1:1 ratio	DEAD added last, solvent CH_2Cl_2	Aziridine formed, MS analysed
PCM435, mixed isomers ~1:1 ratio	DEAD added last, solvent toluene	Aziridine formed, MS analysed

Table 3. Factorial reactions performed.

Mass spectral fragmentation studies were completed on all the reaction products. An example of the fragmentation spectra is found in Figure 4.

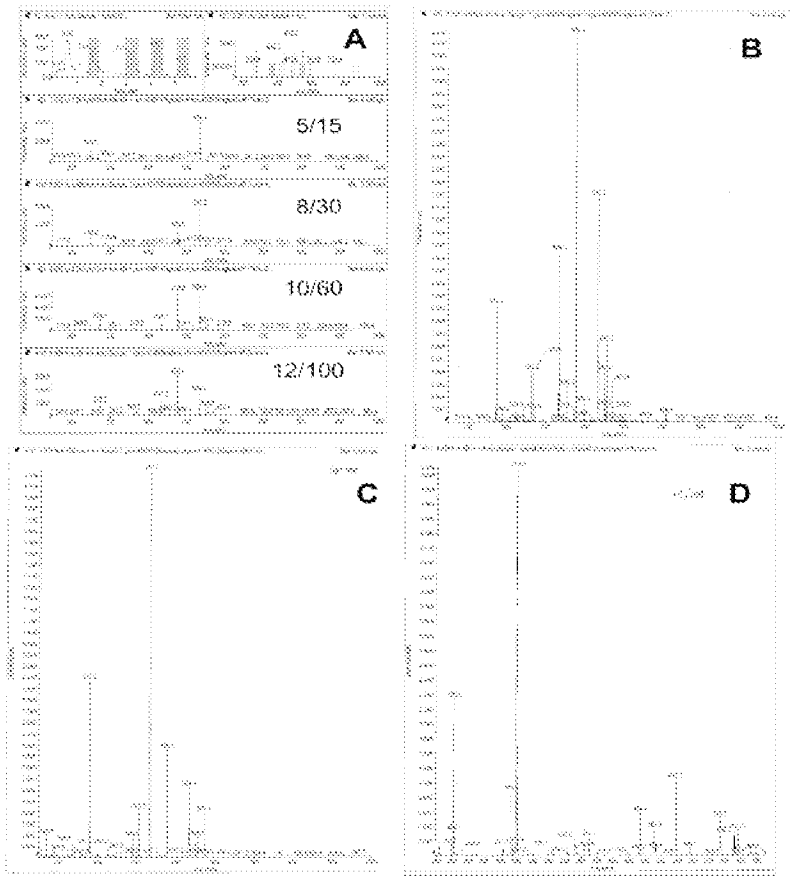


Figure 4. Selected mass spectra. **A** shows mass spectra of azridine **3a** formed in the Mitsunobu cyclisation – the four spectra are at increasing fragmentation energy, EP/DP of 5/15; 8/30; 10/60; 12/100. **B**: an expansion of the **3a** spectrum at 12/100 – note unique fragment ions at 273 and 370. **C**: fragmentation of authentic mimetic **2** at 12/100 – note unique fragment at 277 and absence of 273 and 370 peaks. **D**: spectrum of crude factorial reaction PCM430 at 12/100 – the 279 peak is due to $Ph_3P{=}OH^+$ from the Mitsunobu reagents; note the presence of 273 and 370 - unique fragments from the azridine **3**, and the absence of the 277 peak characteristic of the authentic target **2**.

HPLC Data

Reversed phase HPLC was run on all key compounds and on the crude factorial reaction products. The authentic product **2** and the aziridines show good separation enabling a further check for the formation of **2** to be carried out. None of the crude reactions show evidence of the formation of **2**, while all show the presence of the aziridines **3**. Co-injections were performed to demonstrate that the retention time of **2** is unchanged in the presence of the crude reaction products. Representative traces are included below and all traces are included in the attached materials (the traces below in file HPLC Data 1-compounds and coinjection while the remaining data is file HPLC Data-2).

Compound	10a (alcohol)	10b (alcohol)	2 (authentic target)	3a (aziridine)	3b (aziridine)
Reaction reference	PCM425	PCM402	PCM416	PCM432	PCM414
Retention time (minutes)	8.05	8.11	8.71	6.87	6.93

HPLC Conditions: Agilient 1100 Series LC running Phenomenex Synergi 4 micron column, MAX-RP stationary phase, 50x2.0 mm. Flow rate of 1 ml/min, gradient program 5% to 95% solvent B in 9 minutes. Solvent A: water and 0.05% trifluoroacetic acid; solvent B 10% water in acetonitrile and 0.05% trifluoroacetic acid

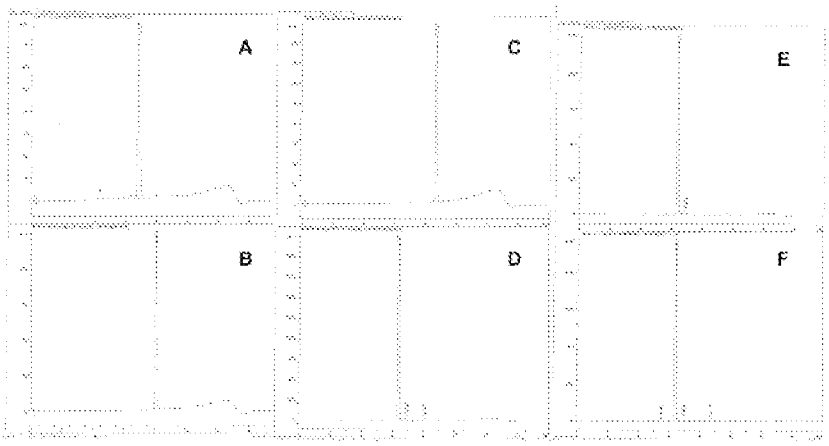


Figure 5. Selected HPLC data. **A** is azirdine **3a** (PCM432); **B** is compound **10a** (PCM425, cyclisation precursor alcohol); **C** is authentic mimetic **2** (PCM416); **D** shows the crude products of reaction PCM430 – main peak at 6.38 is due to triphenylphosphine oxide, peaks for the aziridine and alcohol are present but no trae of compound **2**; **E** crude products of PCM429 showing triphenylphosphine oxide and the aziridine product; **F** is a coinjection of **E** and **C** showing that there is no significant variation in the retention time of **2** when injected as part of the crude reaction product mixture.

mimetics

Experimental Results

Textual listings of spectra for all compounds follow. Copies of all important spectra have been included as attachments. The following table lists the included spectra for each compound.

Table 4. Spectra included as attachments

COMPOUND (DATAFILE)	REACTION	DATA
7 Isoxazoline (Compound 7 PCM403 isoxazoline NMR.pdf)	PCM403	^{13}C, ^{1}H NMR
8 Amino alcohol (Compound 8 PCM411 isomers NMR Mass Spectra.pdf)	PCM 411	^{13}C, ^{1}H NMR
9 Reductive amination product (Compound 9 PCM415-422_NMR_spectra.pdf)	PCM 415 & PCM 422	^{13}C, ^{1}H NMR
10 Alcohol cyclisation precursor (Compound 10 PCM425f7_NMR_andMass_Spectra.pdf)	PCM 425	^{13}C, ^{1}H NMR; COSY TOCSY and fragmentation mass spectrum
3a Aziridine 3 from isomer 1 of 10 (Compound 3a PCM432NMR_MSpectra.pdf)	PCM 432	^{13}C, ^{1}H NMR; COSY TOCSY and fragmentation mass spectrum
3b Aziridine 3 from isomer 2 of 10 (Compound 3b PCM414NMR_MSpectra.pdf)	PCM414	^{13}C, ^{1}H NMR; COSY TOCSY and fragmentation mass spectrum
14 Gamma mimetic, no Cbz (Compound 14 Authentic Mimetic pre Cbz PCM401f7_NMR_spectra.pdf)	401	^{13}C, ^{1}H NMR; COSY TOCSY
2 Authentic target mimetic (Compound 2 Authentic Mimetic PCM416_VtNMR_Mass_spectra.pdf)	416	^{13}C, ^{1}H NMR (variable temp.); COSY TOCSY and fragmentation mass spectrum
Factorial reactions MS analysis (Factorial MS Data PCM427-PCM435.pdf)	PCM427-432	Mass spectra at various fragmentation energies of crude reaction products

Compound Data

Compound 4 (BocIle Weinreb amide)
PCM393
^{1}H NMR (400MHz Varian 298k $CDCl_3$): 5.14 (1H, d, J = 9.7), 4.62 (1H, m), 3.78 (3H, s), 3.22 (3H, s), 1.72 (1H, m), 1.55 (1H, m), 1.43 (9H, s), 1.13 (1H, m), 0.92 (3H, d, J = 6.8), 0.89 (3h, t, J = 7.4).
^{13}C NMR (400MHz Varian 298k $CDCl_3$): 173.1, 155.6, 79.3, 61.4, 54.1, 37.9, 31.8, 28.2 (tBu), 24.2, 15.4, 11.2.

Compound 5 (BocIle aldehyde)
PMC396ede
^{1}H NMR (400MHz Varian 298k $CDCl_3$): 9.66 (1H, s), 5.12 (1H, br), 4.29 (1H, m), 2.02 (1H, m), 1.49 (1H, m), 1.45 (9H, s), 1.27 (1H, m), 0.99 (3H, d, J=6.8Hz), 0.96 (3H, t, J=7.4Hz).
^{13}C NMR (400MHz Varian 298k $CDCl_3$): 200.6, 155.7, (Boc tertiary signal not visible, too few scans), 64.2, 36.4, 28.3 (tBu), 25.3, 15.6, 11.9

Compound 6 (BocIle alkene)
PCM396
^{1}H NMR (400MHz Varian 298k $CDCl_3$): 5.74 (1H, m), 5.17 (1H, m), 5.13 (1H, m), 4.57 (1H, br), 4.09 (1H, br), 1.55 91H, m), 1.47 (1H, m, obscured by tBoc peak), 1.47 (9H, s), 1.11 (1H, m), 0.92 (3H, t, J = 7.3), 0.88 (3H, d, 6.8).
^{13}C NMR (400MHz Varian 298k $CDCl_3$): 155.4, 136.8, 115.2, 79.1, 56.9, 38.9, 28.4 (Boc), 25.3, 15.0, 11.7.

Compound 7 (isoxazoline)
PCM403
In NMR the product peaks were broad at 298K and there was evidence for multiple conformers – some broad double peaks in the carbon spectrum that coalesced at higher temperature. This was confirmed by variable temperature runs
^{1}H NMR (400MHz Varian 318k $CDCl_3$) main isomer (first eluting by flash chromatography): 7.36-7.23 (5H, m), 5.01 (1H, br), 4.39 (1H, br), 3.94 (2H, br), 3.50 (1H, t, J = 8.6 Hz), 2.91 (2H, br), 2.33 (1H, br), 2.05 (1H, brm), 1.53 (2H, m), 1.43 (9H, s), 1.13 (1H, m), 0.91 (3H, d, J = 6.8), 0.87 (3H, t, J = 7.3).
^{13}C NMR (400MHz Varian 318k $CDCl_3$): main isomer 156.5, 137.1, 129.1, 128.3, 127.4, 78.8, 76.2 (br), 61.9 (br), 57.0 (br), 54.0 (br), 38.1, 31.8, 28.4 (Boc), 25.8, 15.8, 11.2.
ISMS: 349.2 (MH^+)

Compound 8 (aminoalcohol)
PCM411
Major isomer (^{13}C NMR (400MHz Varian 298k $CDCl_3$): 156.5, 78.6, 72.5, 59.0, 41.0, 36.7, 34.8, 28.4 (Boc tBu), 25.7, 15.8, 11.3
^{1}H NMR (400MHz Varian 298k $CDCl_3$): 5.00 (1H, d, J = 9.9 Hz), 4.08 (1H, m, Hα), 3.23-3.15 (2H, m), 2.86 (1H, td, J = 12, 3.5 Hz), 1.64-1.53 (3H, m), 1.51 (1H, m), 1.45 (9H, s), 1.13 (1H, m), 0.96 (3H, d, J = 6.7 Hz), 0.89 (3H, t, J = 7.3 Hz)
Minor isomer (second eluting by flash chromatography EtOAc/MeOH/NH_3aq 80:10:5) ^{1}H NMR (400MHz Varian 298k $CDCl_3$): 4.50 (1H, d, J = 10.0 Hz), 3.37 (1H, m), 3.51 (1H, m), 3.20 (1H, m), 2.90 (1H, m), ~2.5 brs H_2O/NH_2, 1.84 (1H, m), 1.72 (1H, m), 1.58 (2H, m), 1.43 (9H, s), 0.96 (1H, m, partially overlapped), 0.92 (6H, m). ^{13}C NMR (400MHz Varian 298k $CDCl_3$): 156.5, 79.1, 73.6, 59.6, 40.6, 34.2, 33.8, 28.4, 23.1, 16.4, 11.8.
ISMS: 261.1 (MH^+)

Compound 9 (reductive amination product)
M415/422
Major isomer (first eluting by flash chromatography EtOAc -> 5% EtOH in EtOAc) ^{13}C NMR (400MHz Varian 298k $CDCl_3$): 171.8, 156.4, 78.7, 72.3, 61.0, 58.8, 50.3, 48.5, 36.8, 32.7, 28.4, 25.7, 15.8, 14.2, 11.3. ^{1}H NMR (400MHz Varian 298k $CDCl_3$): 4.96 (1H, d, J=10.0 Hz), 4.19 (2H, q, J=7.1), 4.05 (1H, m), 3.38 (2H, ABq, J(apparent) 17.4, 14.1 Hz), 3.19 (1H, m), 3.07 (m, 1H), 2.73 (1H, dt, J = 3.1, 11.6), 1.57-1.45 (4H, m), 1.43 (9H, s), 1.28 (3H, t, J = 7.2Hz), 1.13 (1H, m), 0.95 (3H, d, J = 6.7), 0.89 (3H, t, J = 7.3).
Minor isomer ^{13}C NMR (400MHz Varian 298k $CDCl_3$):171.9, 156.5, 79.1, 73.6, 60.9, 59.5, 50.2, 48.1, 34.2, 31.4, 28.4, 23.1, 16.4, 14.2, 11.8.
^{1}H NMR (400MHz Varian 298k $CDCl_3$): (selected peaks from a spectrum of mixed isomers) 4.46 (1H, d, J = 10.2Hz), 4.19 (2H, q, J = 7.1 Hz), 3.74 (1H, m), 3.50 (1H, m), 3.39 (2H, m), 3.06 (1H, m), 2.75 (1H, m), 1.85 (1H, m), 1.72-1.47 (3H, m), 1.43 (9H, s), 1.28 (3H, t, J = 7.1 Hz), 0.98-0.88 (7H, m).
ISMS: 347.0 (MH^+)

Compound 10 (precyclisation alcohol)
PCM425f7 (major isomer, first eluting by flash chromatography EtOAc/light petroleum).
^{1}H NMR (400MHz Varian 298k $CDCl_3$): two rotamers in about 60:40 ratio 7.34 (5H, m, aromatic), 5.65 (0.4H, d, J=8.6Hz), 5.46 (0.6H, d, J=7.6Hz), 5.15-4.98 (2H, benzylic position overlapped pair of ABq, Japp=12.3Hz), 4.86 (1H, NH position overlapped doublets J about 10.2 and 10.6Hz), 4.79 (0.4H, m, Alaα), 4.49 (0.6H, d, 19Hz), 4.44 (0.6H, m, Japp=7.1Hz, Alaα), 4.29 (0.4H, d, 17Hz), 4.23 and 4.18 (2H, 2xq, J=7.1Hz, ethyl ester), 4.13 (1.6H, m, overlapped signals), 3.95 (0.6H, d, 19Hz), 3.85 (0.4H, m), 3.82 (0.4H, 17Hz), 3.75-3.60 (1.2H, m, overlapped), 3.53-3.36 (0.8H, m, overlapped), 3.16 (1H, m), 3.69 (0.4H, m), 2.94 (0.6H, m), 1.93 (0.6H, m), 1.8-1.5 overlapped multiplets and water peak (~4H, m), 1.43 (9H, s), 1.38 (1.2H, d, 6.8Hz), 1.33 (1.8H, d, 6.7Hz), 1.30 (1.2H, t, J=7.2), 1.26 (1.8H, t, J=7.2Hz), 1.13 (1h, m) 0.95-0.83 (6H, overlapped m).
^{13}C NMR (400MHz Varian 298k $CDCl_3$) two rotamers, signals grouped in parentheses where they appear to be from the same carbon according to proximity and relative intensity: (175.5, 173.1), (169.1, 168.9), (156.7, 156.5), (155.8, 155.7), (136.2, 136.1), 128.52, 128.48, 128.2, 128.1, 127.9, (79.3, 78.8), (68.8, 65.7), 66.9, (62.0, 61.3), (59.0, 58.3 49.3, 48.0), (46.7, 46.6), (46.3, 44.6), (36.8, 35.9),
(34.0, 32.3), (28.40, 28.35), (25.7, 25.5), (19.1, 18.4), (15.9, 15.8), 14.1, (11.2, 11.1).
Second eluting isomer: ^{1}H NMR (400MHz Varian 298k $CDCl_3$): 7.33 (5H, m), 5.66 (1H, d, 9.5Hz), 5.16 (1H, d, 12.2Hz), 5.10 (1H, m), 4.96 (1H, d, 12.3Hz), 4.52 (1H, d, 10.3Hz), 4.47 (1H, m), 4.20 (2H, q, 7.2Hz), 3.99 (2H, s), 3.69 (1H, m), 3.50 (1H, m), 3.23 (1H, m), 1.90 (1H, m), 1.80 (1H, m), 1.42 (9H, s), 1.33 (3H, d, 7.0Hz), 1.27 (3H, t, 7.2Hz), 0.93 (6H, m), 0.89 (1H, m).
^{13}C NMR (400MHz Varian 298k CDCl3): 174.0, 169.0, 156.4, 156.3, 135.9, 128.4, 128.1, 128.0, 78.9, 67.0, 66.1, 61.3, 59.6, 47.7, 46.1, 45.3, 34.4, 31.4, 28.3, 22.3, 19.0, 16.4, 14.0, 12.0
ISMS: 552.2 (MH^+); fragmentation mass spectrum at ep/dp 12/100: 590 (7%, MK^+), 574 (7%, MNa^+), 552 (44%, MH^+), 496 (5%, -tBu), 452 (100%, MH^+-Boc), 434 (13%). HPLC: 8.08 min

Compound 14 (BocIle-Ala-Gly-OEt gamma turn mimetic)
^{1}H NMR (400MHz Varian 298k $CDCl_3$): 4.64 (1H, d, J = 10.0 Hz, IleNH), 4.36 (1H, d, J = 17.4, Gly), 4.18 (2H, m, apparent dq, J = 1.2, 7.2, OCH_2 ester), 3.94 (1H, d, J = 17.3, Gly), 3.80 (1H, m, CH_2aN ring), 3.54 (1H, q J = 6.7, AlaHα), 3.32-3.24 (2H, m, IleHα and CH_2bN ring), 3.05 (1H, m, *CH*NH ring), 1.69 (2H, m, CH_2CH_2N), 1.52 (2H, m, Ileβ+γCH_2a), 1.44 (9H, s, Boc), 1.28 (3H, d, 6.6, Alaβ), 1.27 (3H, t, 7.2, ester), 1.13 (1H, m, IleγCH_2b), 0.935 (3H, d, 6.7, Ile γCH_3), 0.90 (3H, t, 7.4, Ile δCH_3).
^{13}C NMR (400MHz Varian 298k $CDCl_3$): 175.5, 169.5, 156.4, 79.0, 61.1, 60.6, 58.6, 54.9, 50.4, 48.9, 36.4, 33.1, 28.3 (Boc), 25.5, 18.9, 15.8, 14.1, 11.2.
ISMS: 400.1 (MH^+)

Compound 2 (authentic target mimetic prepared via conjugate addition and reaction with Cbz-Cl)
Acylation of the sterically hindered ring amine required the use of neat Cbz-Cl and mildly elevated temperature to progress satisfactorily. The product shows multiple conformers in NMR – amide conformers are typical of secondary carbamates but further conformers are also observed possibly due to restricted rotation of the Ile residue due to interaction with the Cbz group. The conformer peaks were observed to coalesce at elevated temperature and resolve at lower temperature by variable temperature NMR confirming that the multiple peaks were from the same compound and not due to different compounds in the sample. The

structure of the product was confirmed by analysis of COSY and TOCSY spectra. These spectra show the isoleucine spin system is intact, specifically the NH resonance occurs at 4.95 ppm, ruling out the N(Cbz, Boc) imide as a possible structure and that the alanine beta proton resonance has moved downfield from 1.28 ppm to 1.65 ppm while the alpha proton resonance has split into two conformers and moved downfield from 3.54 ppm to 5.25 and 5.42 ppm as expected for ring amine acylation.
^{1}H NMR (400MHz Varian **286K** $CDCl_3$): 7.42-7.28 (5H, m, aromatic), 5.44 (~0.3H, m, AlaHα), 5.30-5.19 (~1.6H, m, benzylic and AlaHα), 5.11 (~1.0H, m, benzylic), 4.96 (~0.6H, d, J = 9.9, IleNH), 4.51 (~0.2H, d, J = 9.9, IleNH), 4.47-4.28 (~1.4H, m, ring CH multiplet and Gly doublets), 4.18 (2H, overlapped ester quartets, J=7.2Hz), 4.14 (~0.4H, m, ring CH), 3.94-3.66 (~2H, overlapped Gly doublets and IleHα signals; includes 3.84 d J=17.2Hz), 3.48 (1H, m, ring $CH_{2a}N$), 3.24 (1H, m, ring $CH_{2b}N$), 2.22 (2H, m, ring $\mathbf{CH_2}CH_2N$), 1.7 (3H, m, overlapped alanine doublets J=8Hz), 1.68-1.5 (~2H, m, overlapped Ile β+γ), 1.43 (~4H, s, Boc), 1.36 (~5H, s, Boc), 1.27 (~3H, m, ester CH_3), 1.1 (1H, m, Ile γ), 1.0-0.85 (~5.5H, m), 0.78 (~0.5H, m).
^{13}C NMR (400MHz Varian **286K** $CDCl_3$): peaks are included in parentheses where proximity and relative intensity indicate they are probably from the same carbon; only the two most prevalent conformers have been listed: 173.5, (169.3, 169.1), (158.1, 156.5), 156.2, 155.8), 136.0, 128.8, 128.6, 128.1, 127.6, (79.3, 78.8), (68.5, 68.0), 61.3, (58.8, 58.3), 56.5, 55.4, (51.1, 50.9), (48.7, 48.5), (36.7, 36.4), (30.2, 29.7), 28.3 (Boc), (21.4, 21.0), (19.6, 19.5), (17.1, 17.0), 14.1, (12.1, 11.9).
ISMS: 534.2 (MH^+); fragmentation mass spectrum at dp/ep of 12/109: 572 (11%, MK^+), 556 (14%, MNa^+), 551 (5%, MNH_4^+), 534 (21%, MH^+), 478 (24%, -tBu), 434 (100%, -Boc), 406 (19%), 277 (26%).

Compound 3 (aziridine Mitsunobu product)

Note on comparison of products 3a and 3b to literature N-Boc-aziridines

The chemical shift data for the protons and carbons of the aziridine ring in products **3** were compared to literature data and found to shown good correspondence. For example in six Boc-aziridines with 2,3 alkyl substituents reported by Righi et al (*Tetrahedron* **2001**, 57, 10039-10046) the aziridine carbamate carbon was found at 160.6 to 161.8 ppm compared to 160.8 and 162.7 for **3**. Righi report the aziridine ring CH signals from 2.38-1.95 compared with 2.18, 2.13, 2.05 and 2.01 for **3**. These signals are in contrast to authentic **2** where the equivalent IleHα shift is at 3.9 ppm, a particularly clear indication of the different structures, along with the absence of the IleNH signal.

3a PCM432 (formed from first eluting isomer of compound **10**)
^{1}H NMR (400MHz Varian 298k $CDCl_3$) two conformers observed in the ratio of about 2:1. The spectrum was assigned based on COSY and TOCSY results, all spectra are included in the appendix: 7.34 (5H, m), 5.67 (1H, overlapped AlaNH doublets), 5.09 (2H, overlapped benzylic), 4.73 (0.7H, AlaαH major conformer, apparent pentuplet, J~7Hz), 4.48 (0.3H, apparent pentuplet, J~7Hz, AlaαH minor conformer), 4.42 (0.3H, d, J=18.4Hz GlyHα), 4.35-4.13 (3H, overlapped quartets and doublets from OCH_2 and GlyHα), 3.84 (0.7H, d, 17.1Hz GlyHα), 3.69 (1H, m, NCH_{2a}), 3.51 (1H, m, NCH_{2b}), 2.39 (1H, m, $N\mathbf{C}H\mathbf{C}H_2$ aziridine), 2.18 (0.7H, dd, J=9.8, 6.8Hz, IleHα aziridine ring), 2.13 (0.3H, dd, J=9.8, 6.6Hz, IleHα aziridine ring), 2.00 (1H, m, $NCH_2C\mathbf{H}_{2a}$), 1.71 (1H, m, $IleγCH_{2a}$), 1.5 (1H, m, partially obscured by tBu, $NCH_2C\mathbf{H}_{2b}$), 1.43 & 1.44 (9H, s, tBu), 1.41 (~2H, d, J=7.1, Alaβ

major conformer), ~1.39 (1H, m, overlapped signal, IleγCH$_{2b}$), 1.34 (~2H, t, J=7.1, ester CH$_3$ major conformer), 1.32 (~1H, d, J=6.7Hz, AlaHβ minor conformer), 1.26 (1H, t, J=7.1, ester CH$_3$ minor conformer), 1.16 (1H, m, Ileβ), 0.98 (3H, overlapped triplets, J~7.5Hz, IleδCH$_3$), 0.90 (3H, overlapped doublets, J~7Hz, IleγCH$_3$).
^{13}C NMR (400MHz Varian 298k CDCl$_3$) two conformers, peaks arising from the same carbon are included in parentheses where they appear to be from the same carbon according to proximity and relative intensity: (173.1, 173.0), (169.2, 168.8), (162.7, 162.4), 155.5, 136.4, 128.5, 128.1, 128.0, (81.1, 80.9), 66.7, 64.1, (61.8, 61.2), (50.4, 48.2), 48.0, (47.8, 47.5), (47.3, 46.8), (39.7, 39.1), (34.0, 33.8), (28.0, 27.9), (27.5, 25.9), (19.3, 19.0), (16.3, 16.2), (14.12, 14.09), (10.8).
ISMS: 534.2 (MH^+); fragmentation mass spectrum at dp/ep of 12/100: 572 (8%, MK^+), 556 (15%, MNa^+), 552 (9%, MH_3O^+), 534 (40%, MH^+), 496 (4%, MH_3O^+-tBu), 478 (68%, MH^+-tBu), 434 (30%, MH^+-Boc), 370 (8%), 363 (9%), 273 (21%).

3b PCM414 (formed from second eluting isomer of compound **10**)
^{1}H NMR (400MHz Varian 298k CDCl$_3$) two conformers observed in the ratio of about 2:1. 7.24 (5H, m), 5.60 (1H, d, 8Hz, AlaNH), 5.09 (2H, m, benzylic), 4.71 (0.7H, m, AlaHα), 4.46 (0.3H, m, AlaHα), 4.32 (~2H, q, J=7.0), 4.28-4.15 overlapped signals from OCH$_2$ and Gly including 4.17 (q, J=7), 4.02 (0.3H, d, J=18.5), 3.86 (0.7H, d, J=17.1), 3.75 (1H, m, NCH$_{2a}$), 3.53 (1H, m, NCH$_{2b}$), 2.18 (1H, m, OCH), 2.05 (0.7H, dd, J=3.5, 8.1Hz, IleHα), 2.01 (0.3H, dd, J=3.4, 8.1Hz, IleHα), 1.89 (1H, m, NCH$_2$C**H**$_{2a}$), 1.70 (2H, m, IleγCH$_{2a}$ and NCH$_2$C**H**$_{2b}$), 1.454 and 1.450 (9H, s), 1.40 (~2H, d, J=6.8Hz Alaβ major conformer), 1.34 (t, 7.1Hz, major conformer), 1.26 (t, J=7.2Hz, minor conformer), 1.13 (1H, m, Ileβ), 0.93 (6H, m, Ile γ&δ CH$_3$).
^{13}C NMR (400MHz Varian 298k CDCl$_3$): 173.3, 168.9, 160.8, 155.5, 131.8, 128.5, 128.1, 128.0, (81.2, 81.0), 66.7, 64.1, (62.6, 62.3), (61.9, 61.3), (49.7, 49.0), 48.3, 46.9, 46.8, 46.2, (40.0, 39.2), 36.8, (31.1, 29.1), (27.95, 27.9), (27.44, 27.39), (19.3, 19.2), (16.3, 16.2), 14.1, 11.0.
ISMS: 534.2 (MH^+); fragmentation mass spectrum at dp/ep of 12/100: 572 (19%, MK^+), 556 (36%, MNa^+), 552 (36%, MH_3O^+), 534 (35%, MH^+), 496 (20%, MH_3O^+-tBu), 478 (84%, MH^+-tBu), 434 (67%, MH^+-Boc), 370 (30%), 363 (26%), 273 (100%)

mimetics

Appendix 1

Comparison of Ms data (unassigned mixture) with assigned spectra of actual product isomers and authentic mimetic.

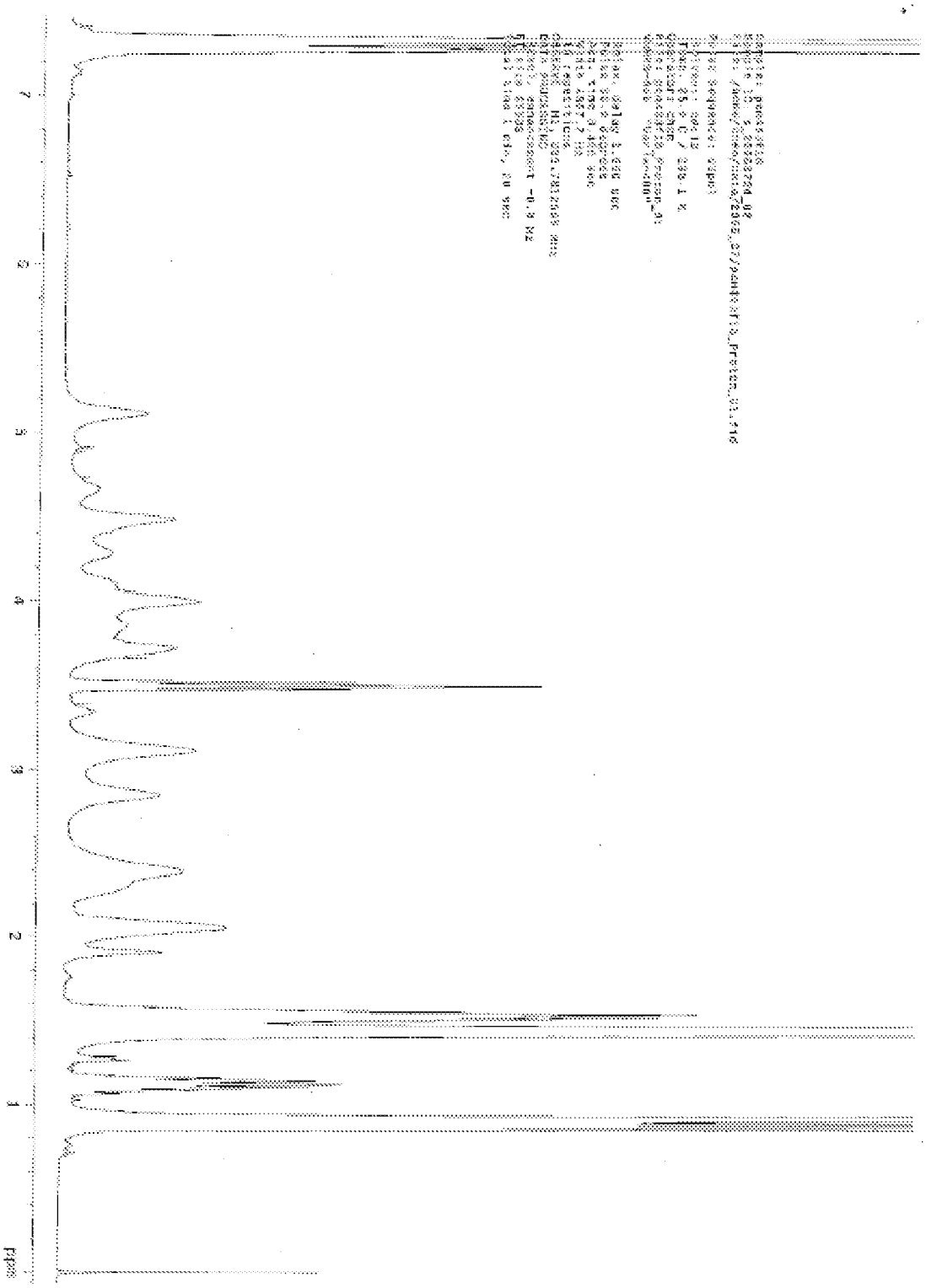
Ms data for mixed epimers[a]	PC31432 (aziridine 3a)	PC31414 (aziridine 3b)	PC31416 (authentic 2)
7.28 5H, s	7.34 (5H, m),	7.34 (5H, m)	7.42-7.28 (5H, m)
5.65-5.52 1H, m	5.67 (1H, overlapped AlaNH doublets)	5.60 (1H, d, 8Hz, AlaNH)	
			5.44 (0.3H, m, AlaHα 5.36-5.19 (1.6H, m, benzyl and AlaHα), 5.11 (1H, m, benzyl)
5.05 2H, s	5.09 (2H, overlapped benzylic)	5.09 (2H, m, benzylic)	
	4.73 (0.7H, AlaαH major conformer, apparent pentuplet, J~7Hz)	4.71 (0.7H, m, AlaHα)	4.96 (0.5H, d, J = 9.9, Ile NH major conformer)
	4.48 (0.3H, apparent pentuplet, J~7Hz, AlaαH minor conformer)	4.46 (0.3H, m, AlaHα)	4.51 (0.2H, d, J = 9.9, IleNH minor)
	4.42 (0.3H, d, J=18.4Hz GlyHα),		4.47-4.28 (1.4H, m, ring CH and Gly doublets)
4.35-4.03 2H, m	4.35-4.13 (3H, overlapped quartets and doublets from OCH_2 and GlyHα),	4.32 (~2H, q, J=7.0) 4.28-4.15 overlapped signals from OCH_2 and Gly including 4.17 (q, J=7)	4.18 (2H, m, overlapped ester OCH_2 quartets), 4.14 (0.4H, m, ring CH)
		4.02 (0.3H, d, J=18.5), 3.86 (0.7H, d, J=17.1)	3.94-3.68 (2H, Gly and IleHα overlapped
3.75-3.35 2H, m	3.69 (1H, m, NCH_{2a}), 3.31 (1H, m, NCH_{2b})	3.75 (1H, m, NCH_{2a}), 3.53 (1H, m, NCH_{2b})	3.48 (1H, m, ring $CH_{2a}N$)
			3.24 (1H, m, ring $CH_{2b}N$)
2.4-2.25 1H, m	2.39 (1H, m, NCH aziridine),		
2.18-2.0 1H, m	2.18 (0.7H, dd, J=9.8, 6.8Hz, IleHα), 2.13 (0.3H, dd, J=9.8, 6.6Hz, IleHα),	2.05 (1H, m, NCH azridine), 2.01 (0.3H, dd, J=3.4, 8.1Hz, IleHα), 2.05 (0.7H, dd, J=3.5, 8.1Hz, IleHα),	
	2.06 (1H, m, NCH_2CH_{2a})	1.89 (1H, m, NCH_2CH_{2a})	
	1.71 (1H, m, $Ile_\gamma CH_{2a}$)	1.70 (2H, m, $Ile_\gamma CH_{2a}$ and NCH_2CH_{2a})	1.7 (3H, m, overlapped alanine doublets J=8Hz)
1.58 2H, s			1.65-1.5 (2H, m, overlapped Ile_γ)
1.5-1.45 1H, m	1.5 (1H, m, partially obscured by tBu, NCH_2CH_{2b}),		
1.45 9H, s	1.43 & 1.44 (9H, s, tBu)	1.454 and 1.450 (9H, s)	1.43 (4H, s), 1.36 (5H, s) Boc
	1.41 (~2H, d, J=7.1, Alaβ major conformer), ~1.39 (1H, m, overlapped signal	1.40 (~2H, d, J=6.8Hz Alaβ major conformer),	
1.35-1.0 9H, m	1.34 (~2H, t, J=7.1, ester CH_3 major conformer), 1.32 (~1H,	1.34 (2H, t, 7.1Hz, major conformer), 1.26 (1H, t, J=7.2Hz, minor	1.27 (3H, m, ester) 1.1 (1H, m, Ileβ)

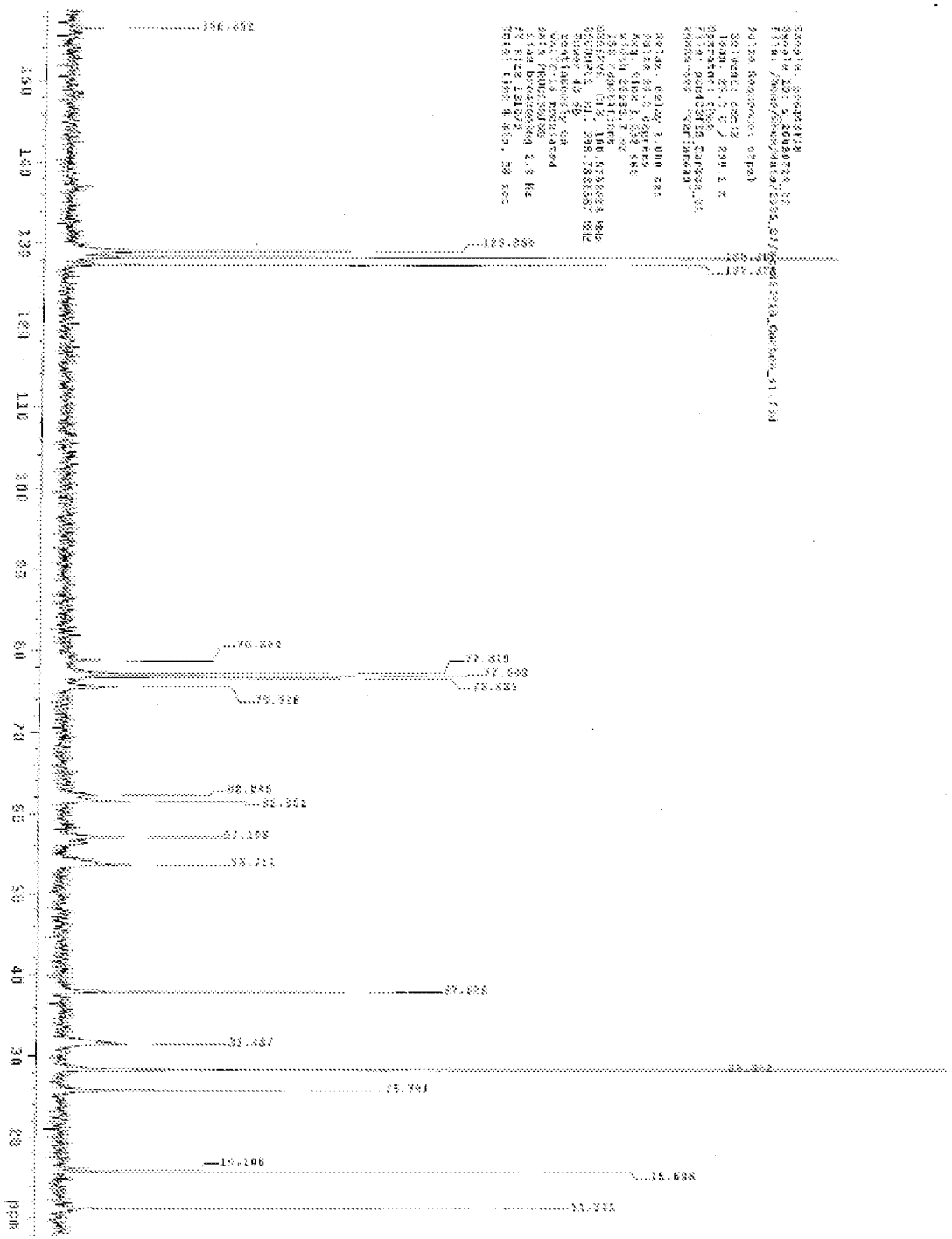
	d, J=6.7Hz, AlaHβ minor conformer), 1.26 (1H, t, J=7.1, ester CH_2 minor conformer), 1.16 (1H, m, Ileβ)	conformer), 1.13 (1H, m, Ileβ)	
0.98-0.74 8H, m	0.98 (3H, overlapped triplets, J~7.5Hz, IleδCH_3), 0.90 (3H, overlapped doublets, J~7Hz, IleγCH_3)	0.93 (6H, m, Ile γ& δ CH_3)	1.0-0.85 (5.5H, m), 0.78 (0.5H, m) Ile CH_3

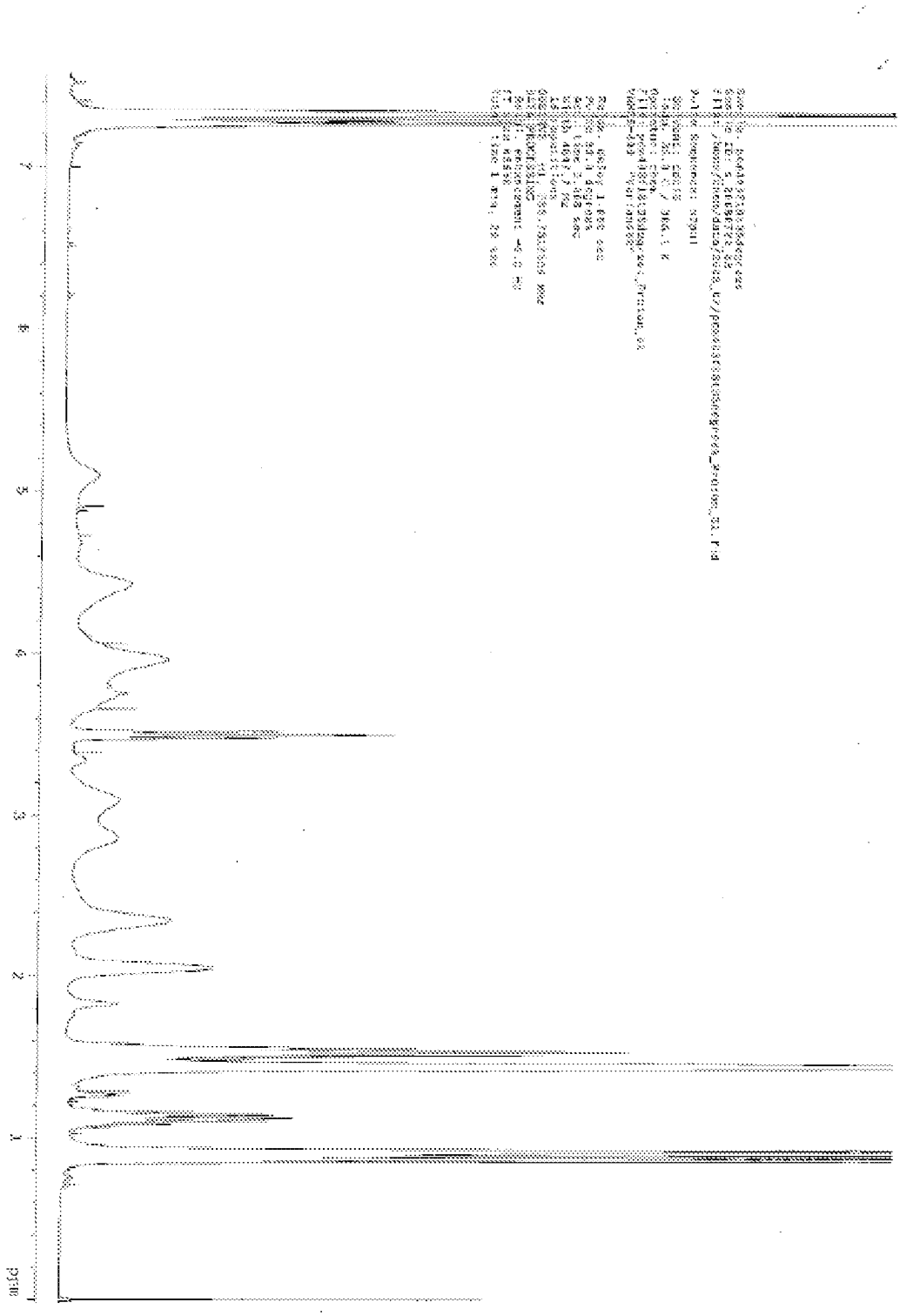
*No epimer ratio was specified in the publication

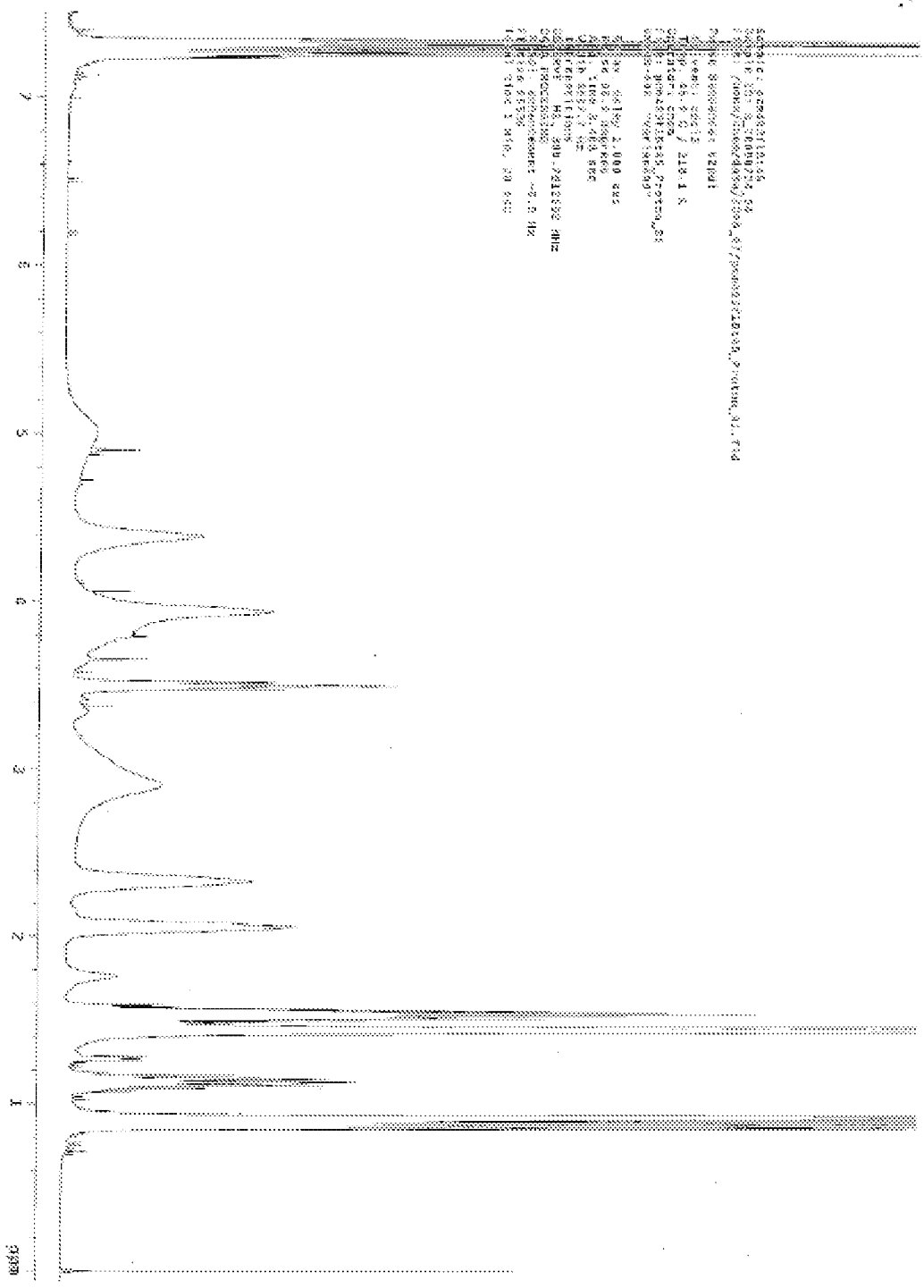
Appendix 2

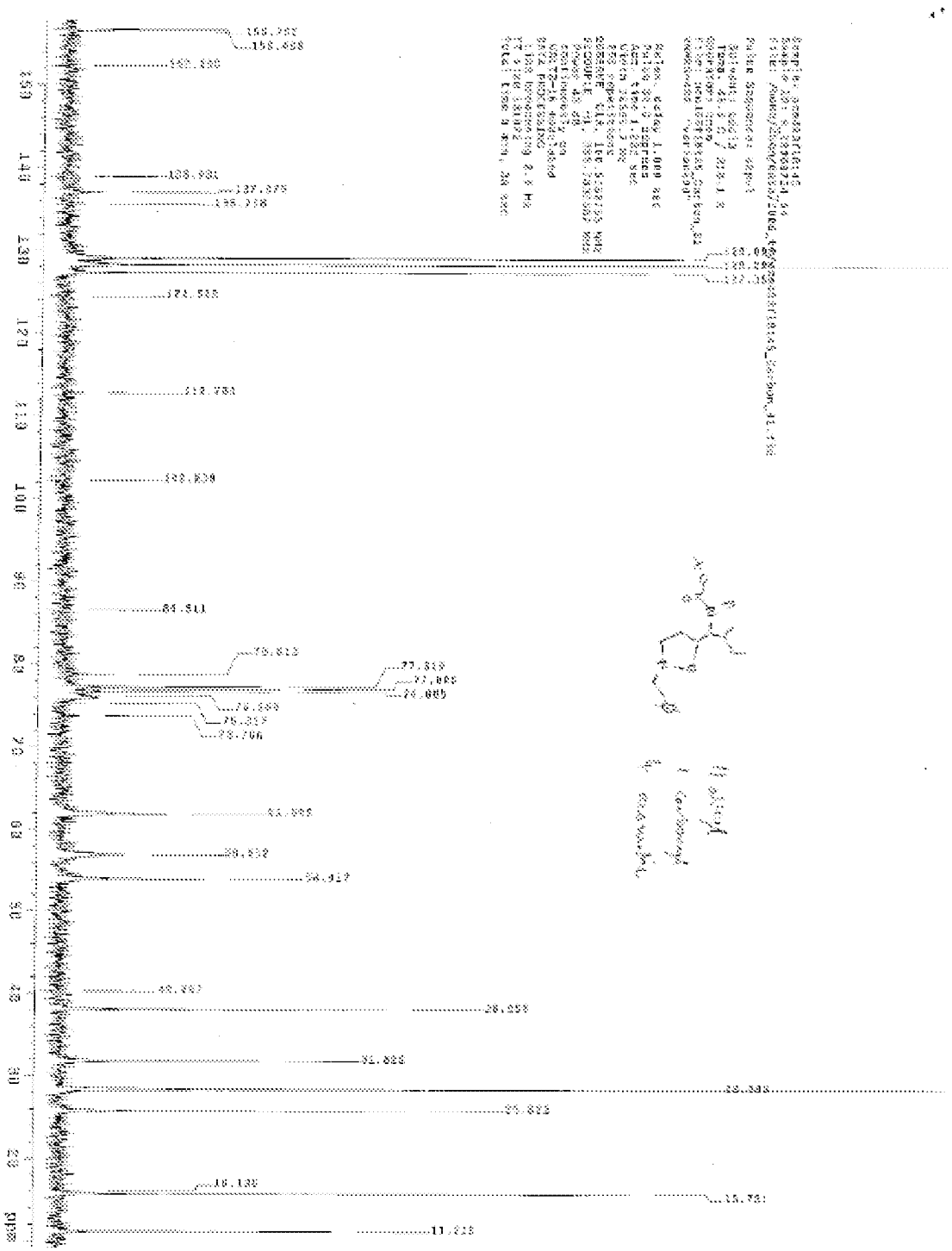
Data obtained for Compounds 7





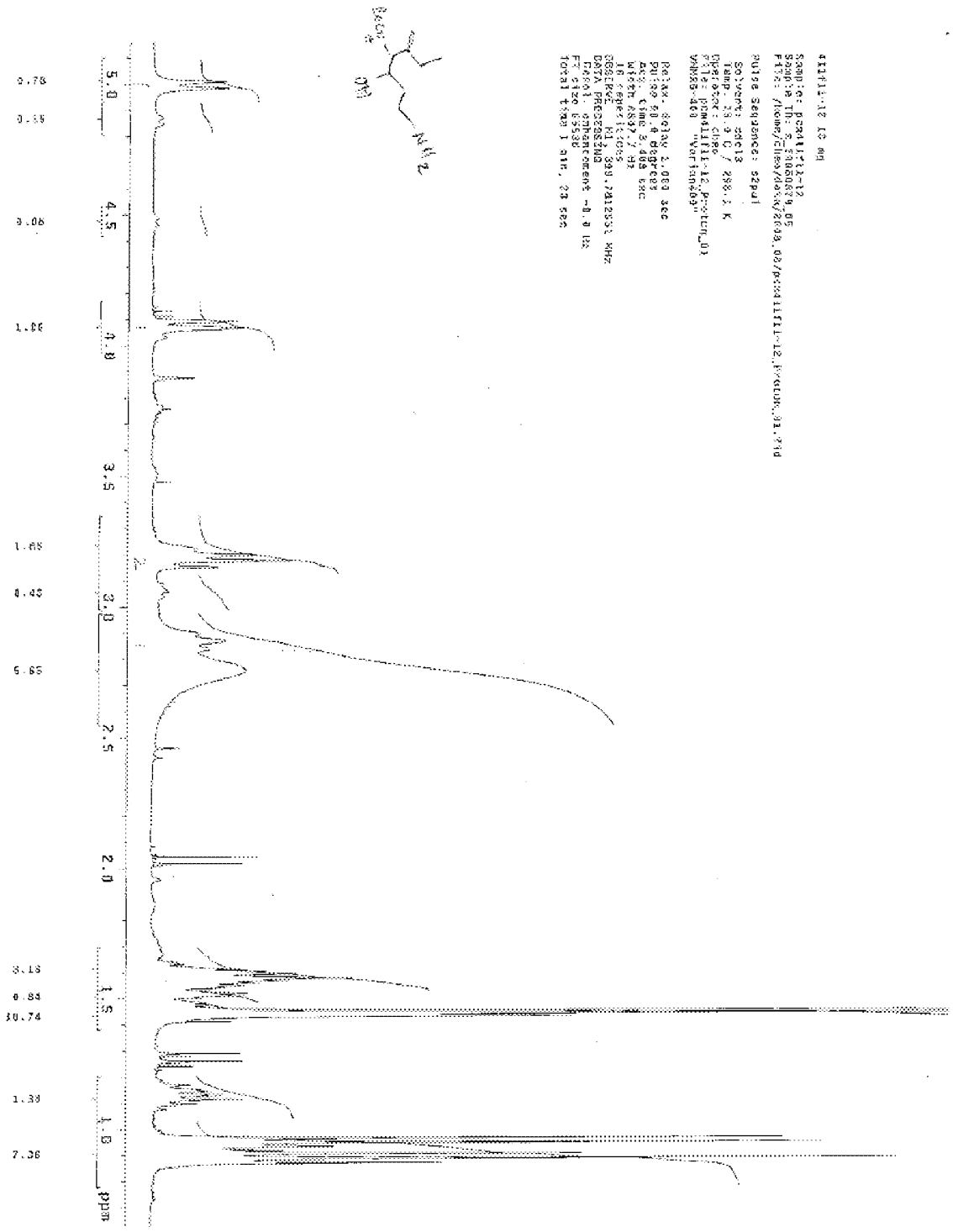


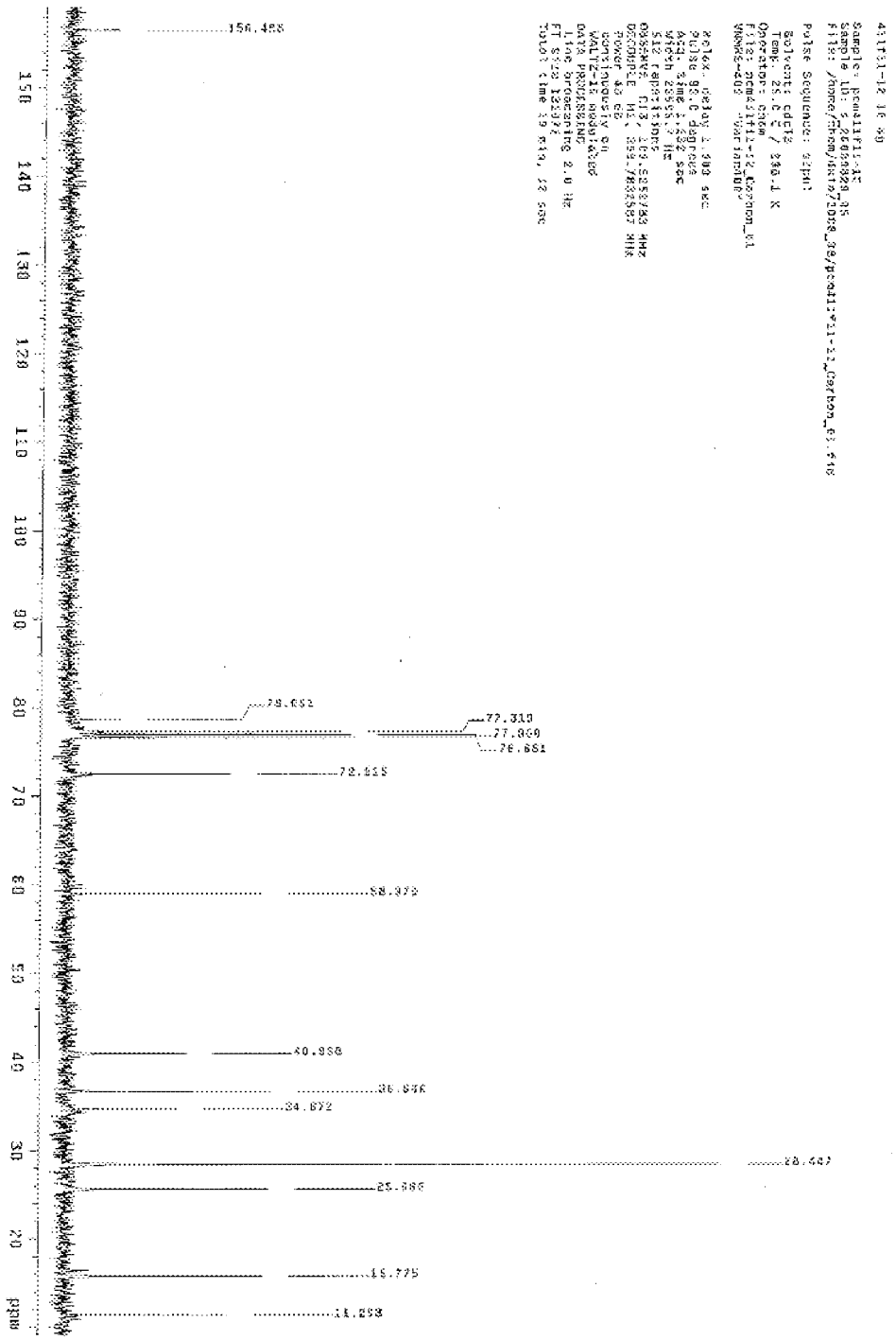


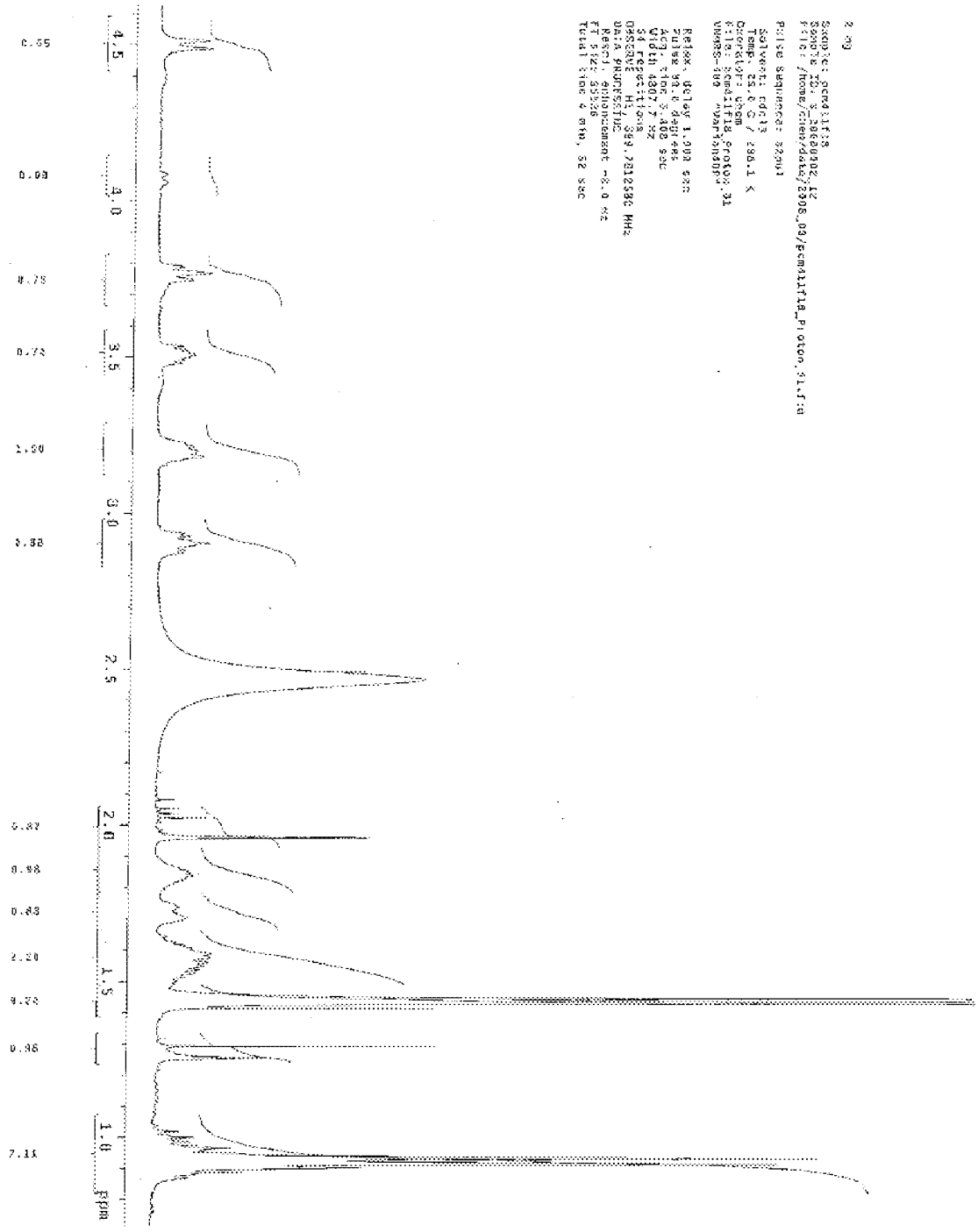


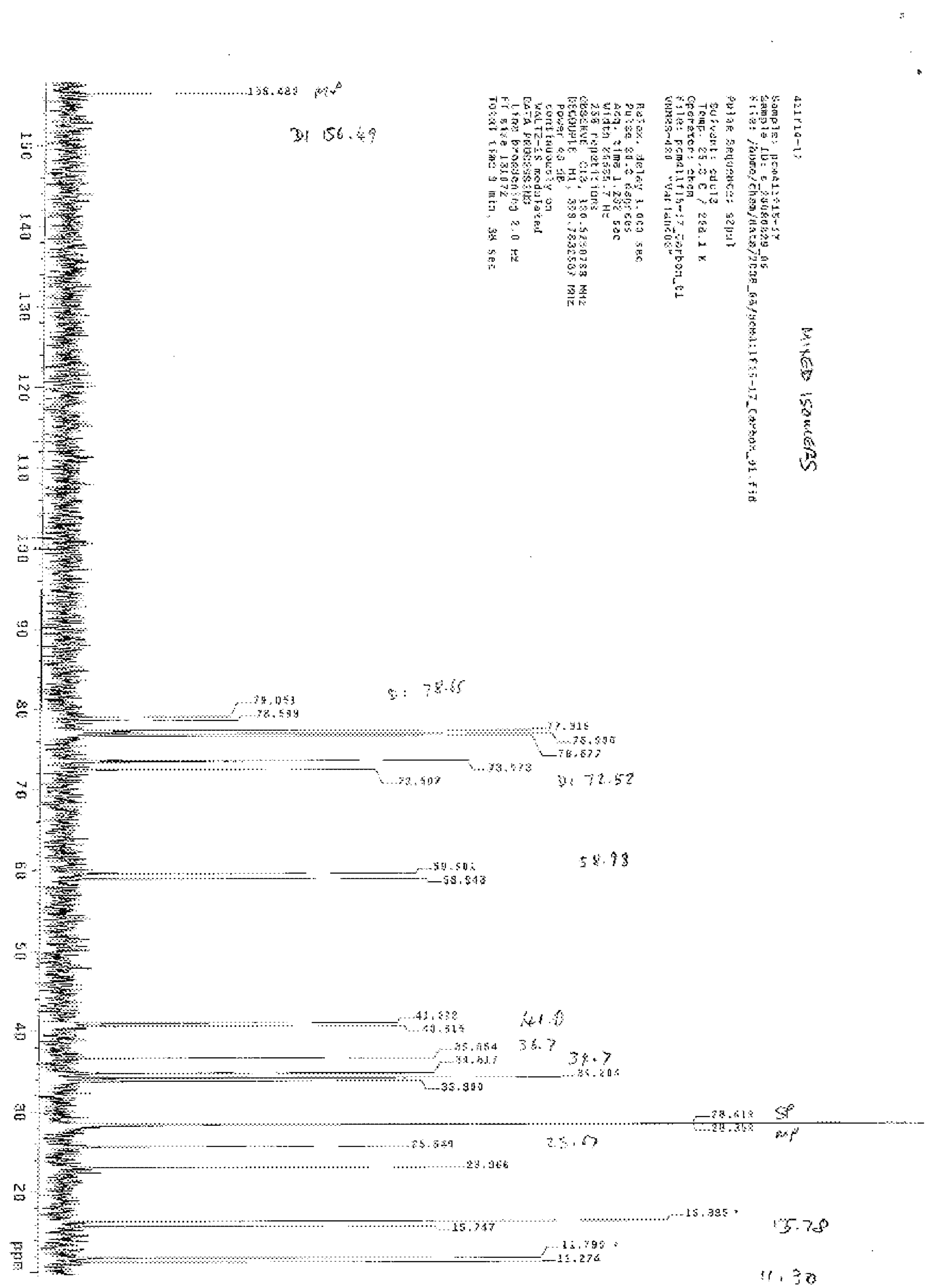
Appendix 3

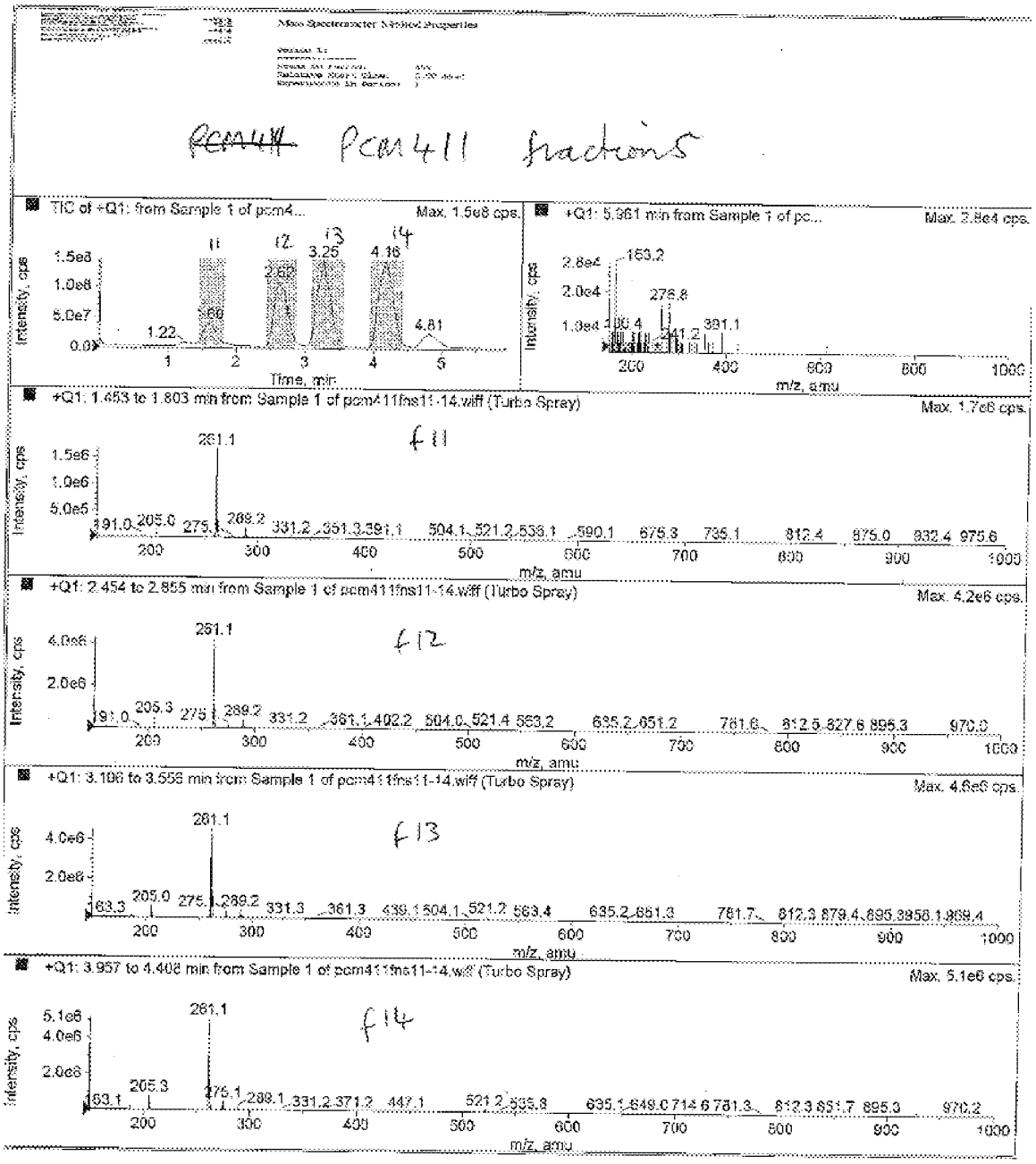
Data obtained for Compounds 8


0.78
0.86
3.06
1.86
1.68
0.45
5.65
3.18
0.84
30.74
1.38
7.36
5.0
4.5
4.0
3.5
2.0
2.5
2.0
1.5
1.0
ppm


156.488
79.652
77.319
77.000
76.681
72.415
58.972
40.988
36.646
34.672
28.462
25.886
15.775
11.253
ppm

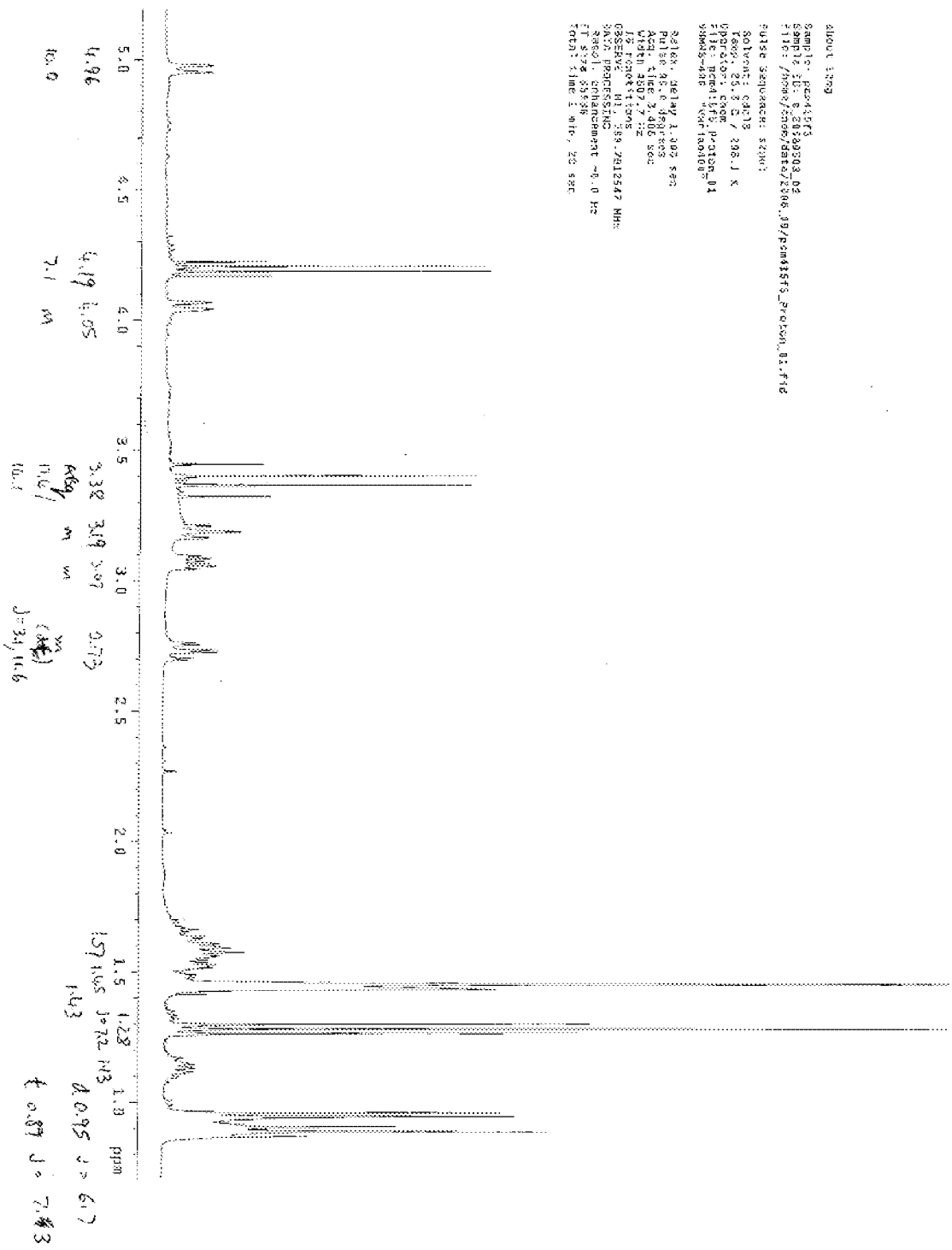

6.65
0.93
4.78
0.78
1.80
3.82
9.87
8.98
8.83
2.20
9.78
8.96
7.11
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

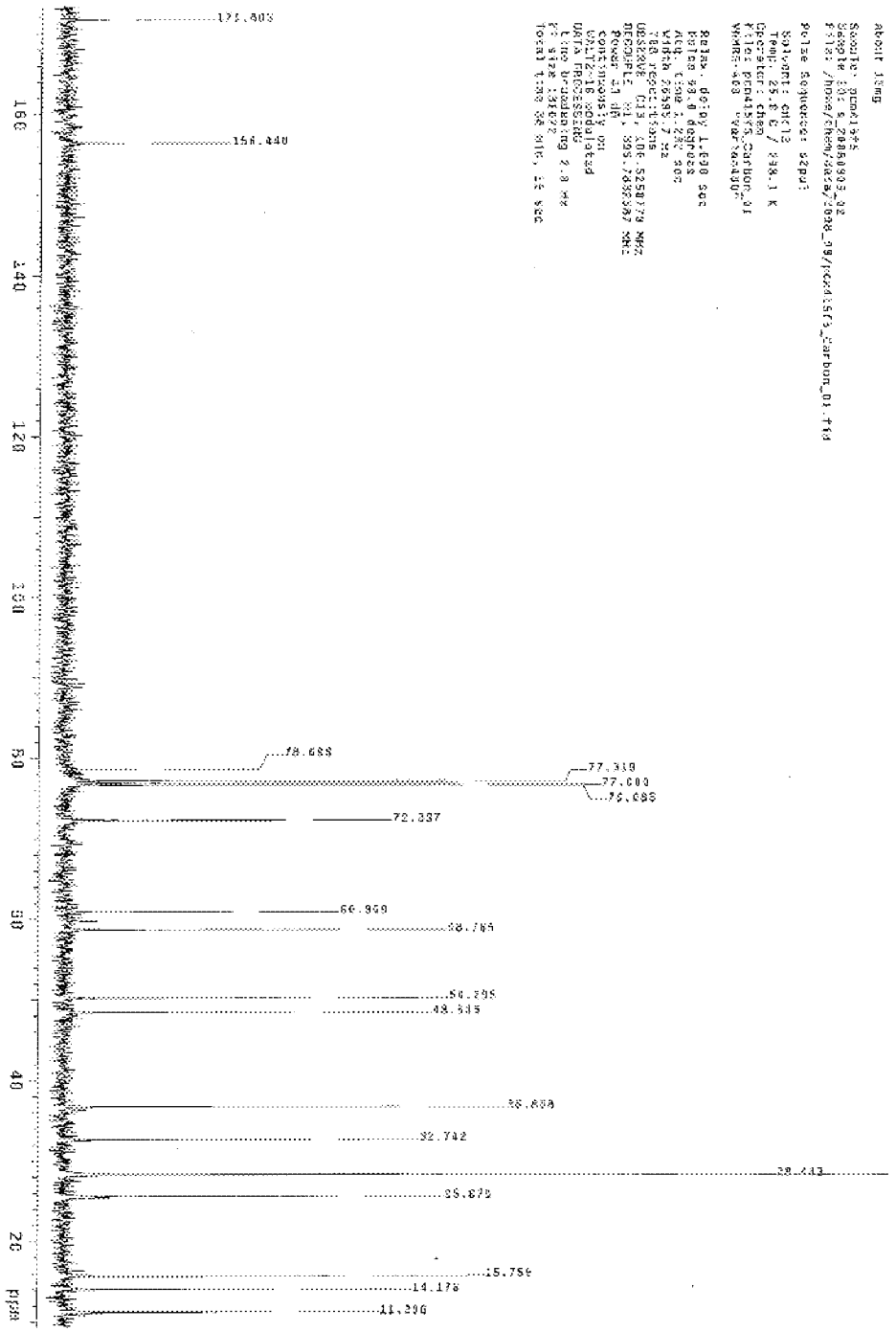


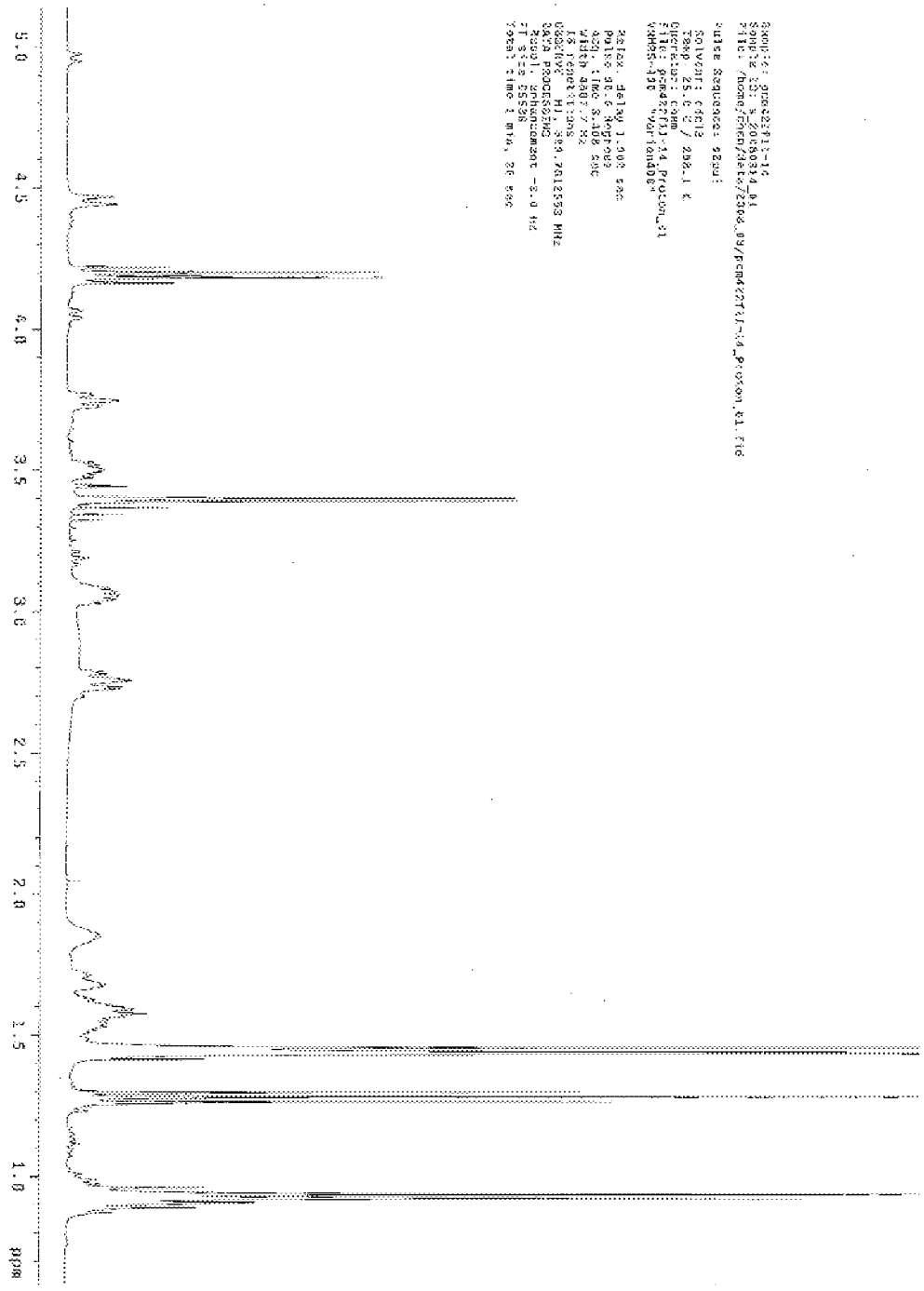

Mass Spectrometer Method Properties
~~PCM411~~ PCM411 fractions
TIC of +Q1: from Sample 1 of pcm4...
Max. 1.5e8 cps.
Intensity, cps
Time, min
f1
f2
f3
f4
3.25
4.16
1.22
4.81
+Q1: 5.361 min from Sample 1 of pc...
Max. 2.8e4 cps.
163.2
276.8
391.1
m/z, amu
+Q1: 1.453 to 1.803 min from Sample 1 of pcm411fns11-14.wiff (Turbo Spray)
Max. 1.7e6 cps.
f11
261.1
+Q1: 2.454 to 2.855 min from Sample 1 of pcm411fns11-14.wiff (Turbo Spray)
Max. 4.2e6 cps.
f12
261.1
+Q1: 3.196 to 3.556 min from Sample 1 of pcm411fns11-14.wiff (Turbo Spray)
Max. 4.6e6 cps.
f13
261.1
+Q1: 3.957 to 4.408 min from Sample 1 of pcm411fns11-14.wiff (Turbo Spray)
Max. 5.1e6 cps.
f14
261.1

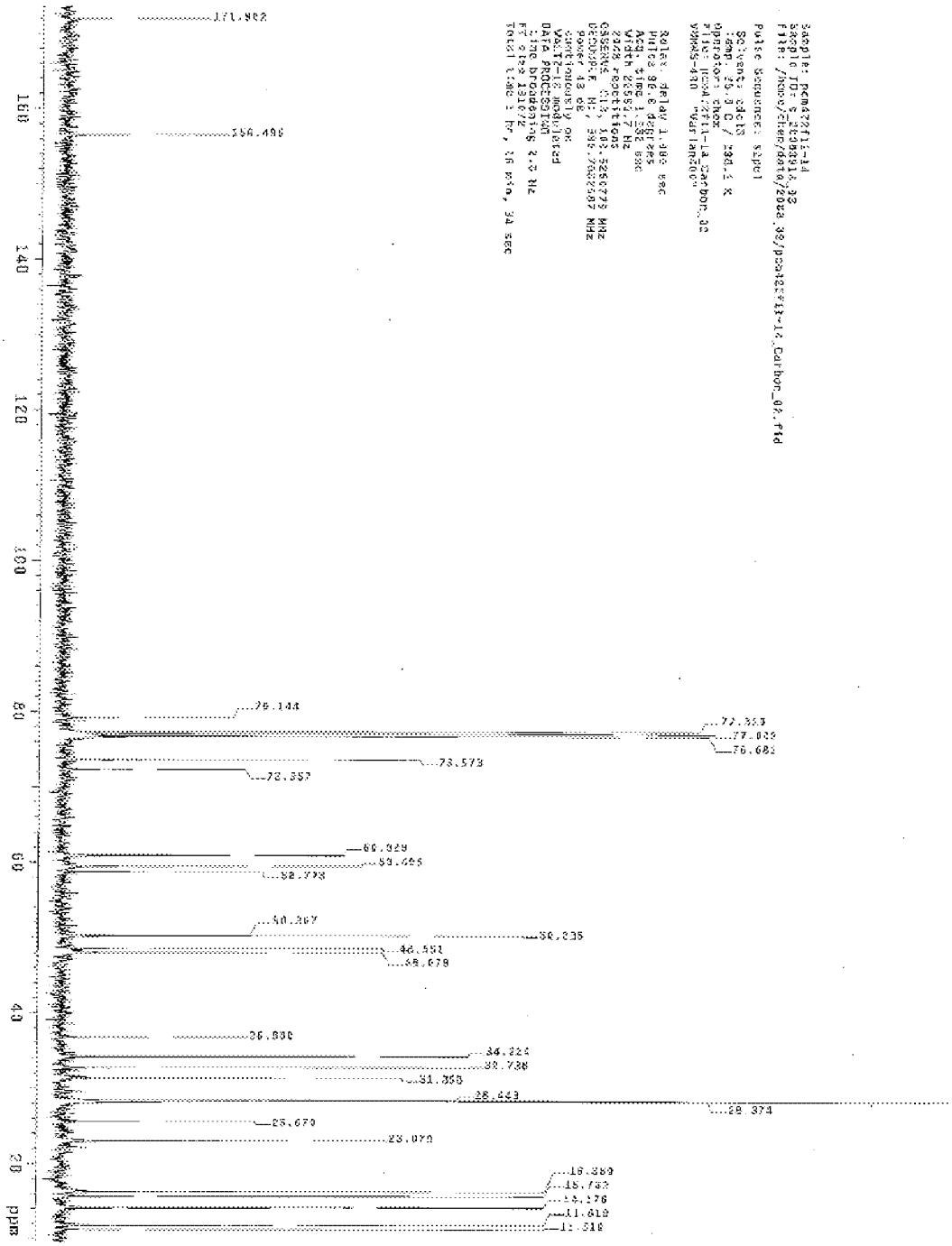
Appendix 4

Data obtained for Compounds 9


5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

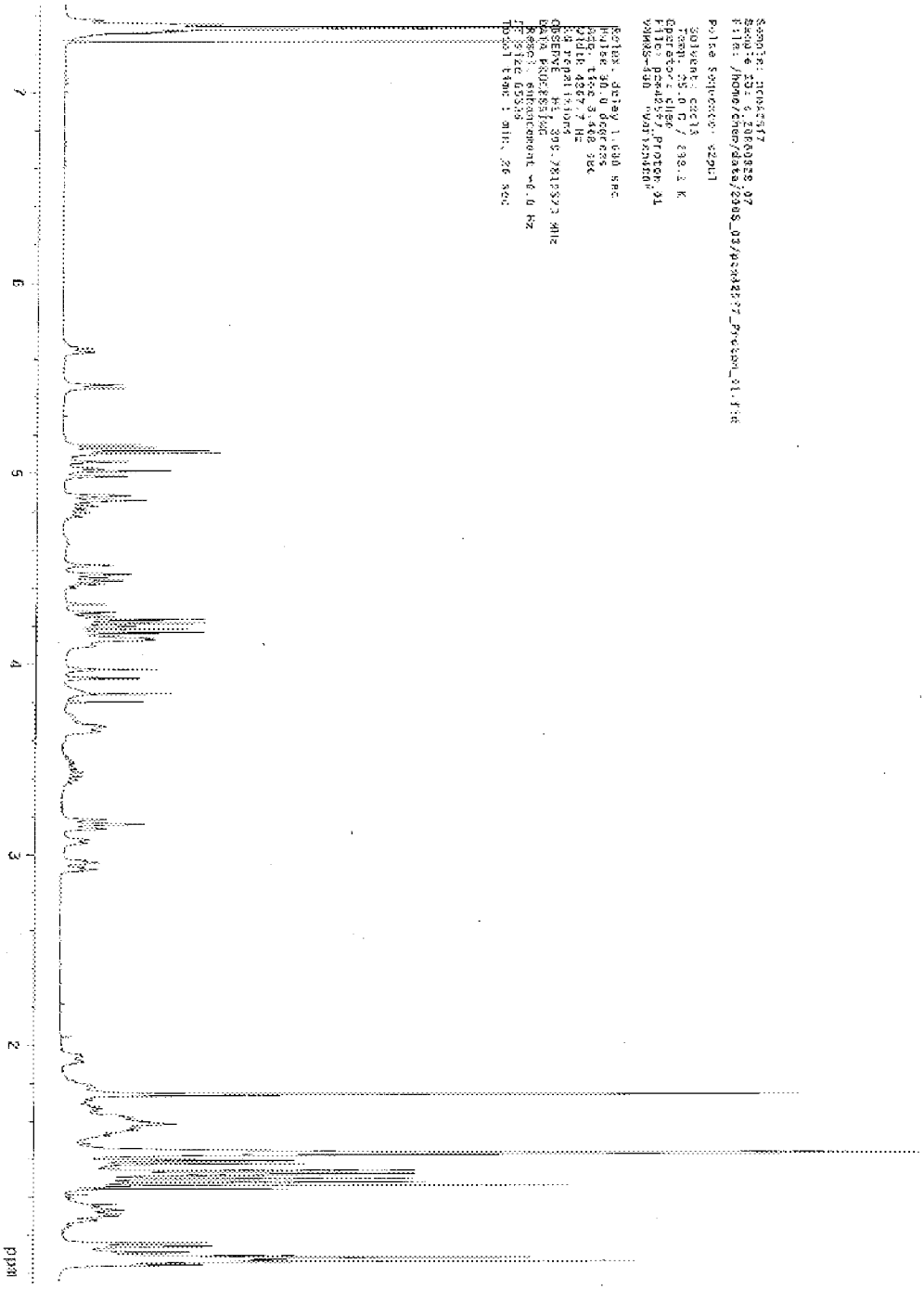

171.803
156.440
78.688
77.310
77.000
76.695
72.337
66.959
58.765
56.795
48.535
38.858
32.742
30.443
25.879
15.756
14.176
11.296
160
240
120
200
80
40
20
PPM

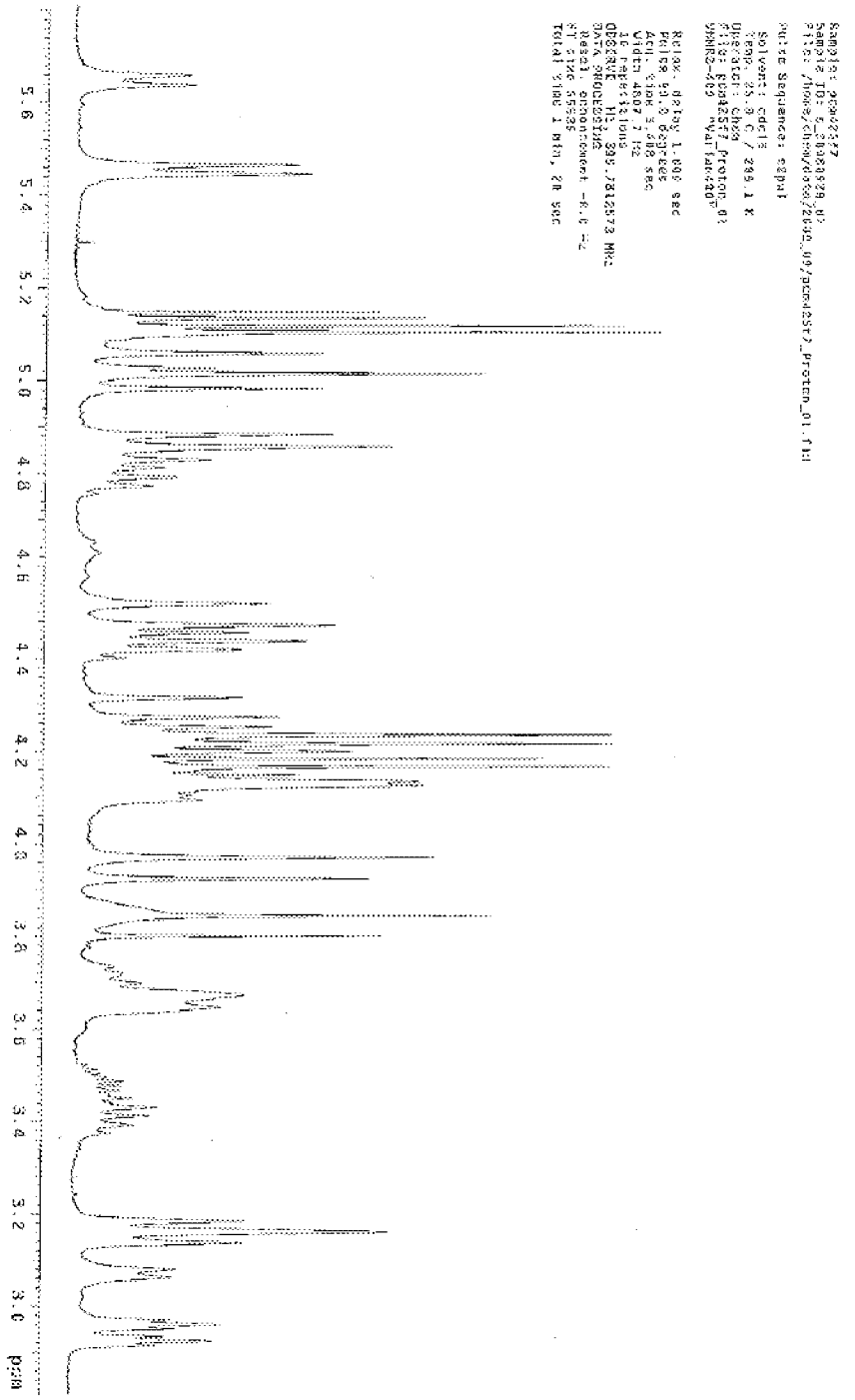

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
PPM

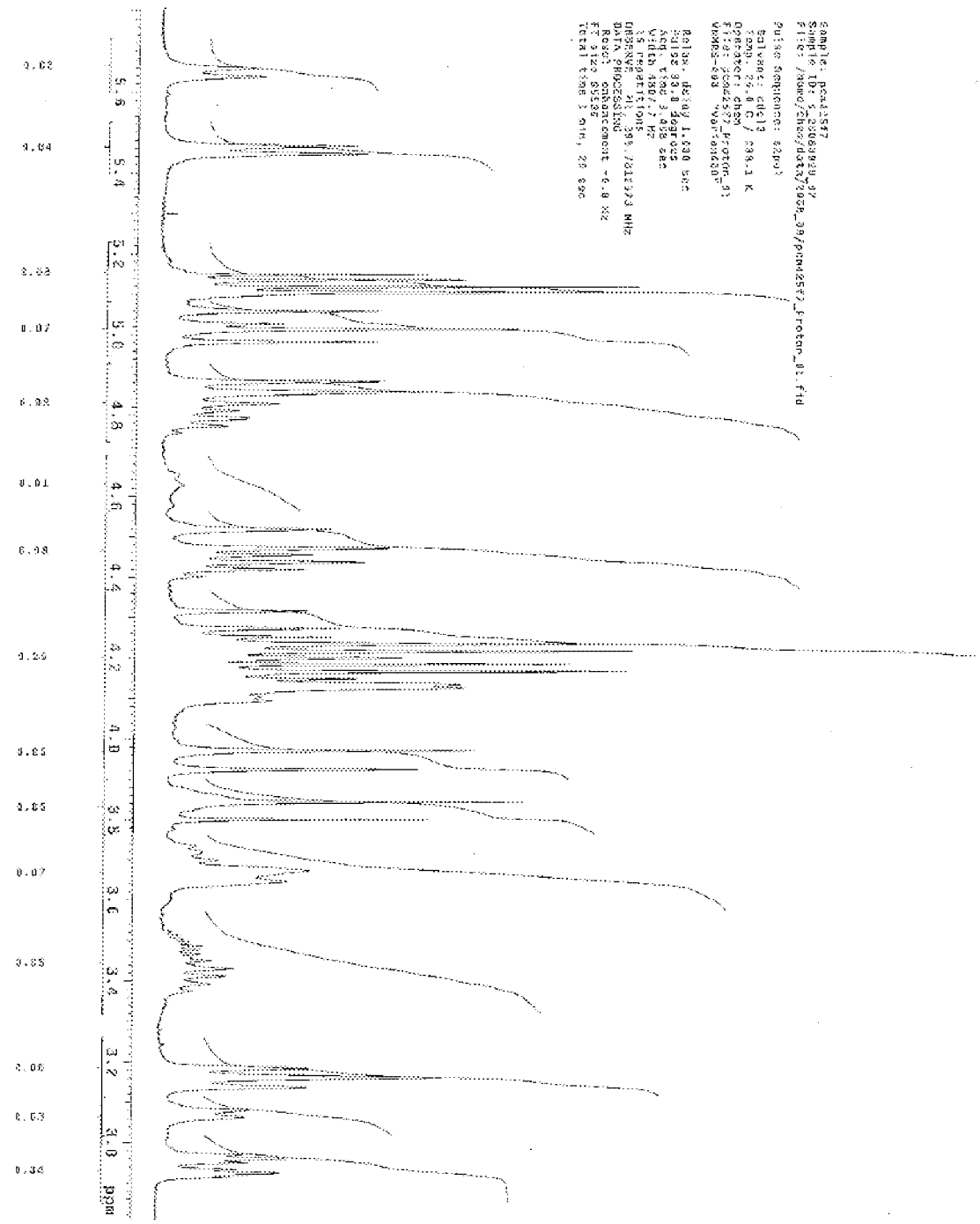

171.982
156.496
79.144
77.353
77.029
76.682
73.573
72.357
66.929
63.695
62.798
60.267
58.235
46.551
44.678
36.830
34.224
32.738
31.355
28.443
28.374
25.679
23.070
16.389
15.753
14.176
11.612
11.518
ppm

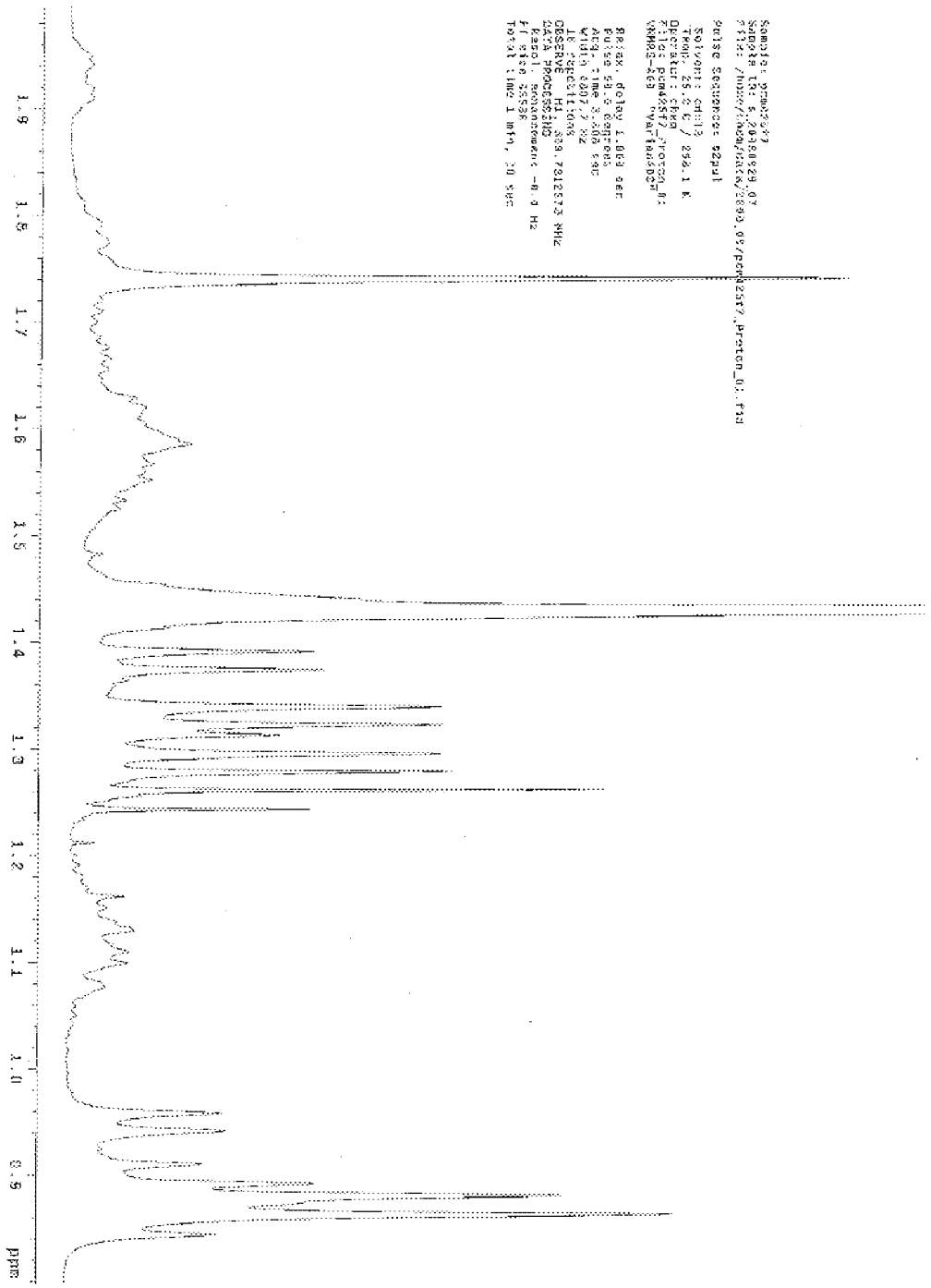
Appendix 5

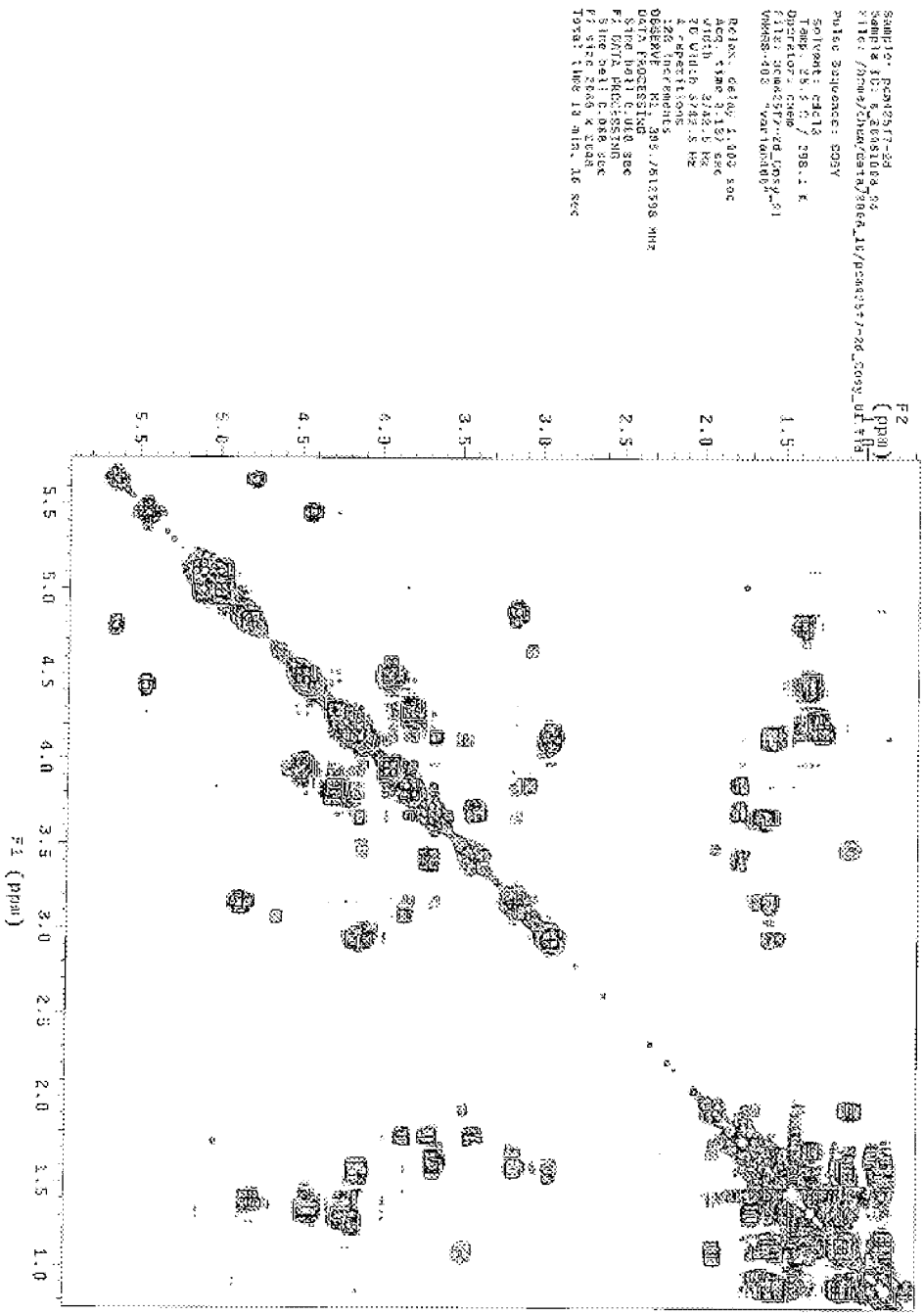
Data obtained for Compounds 10

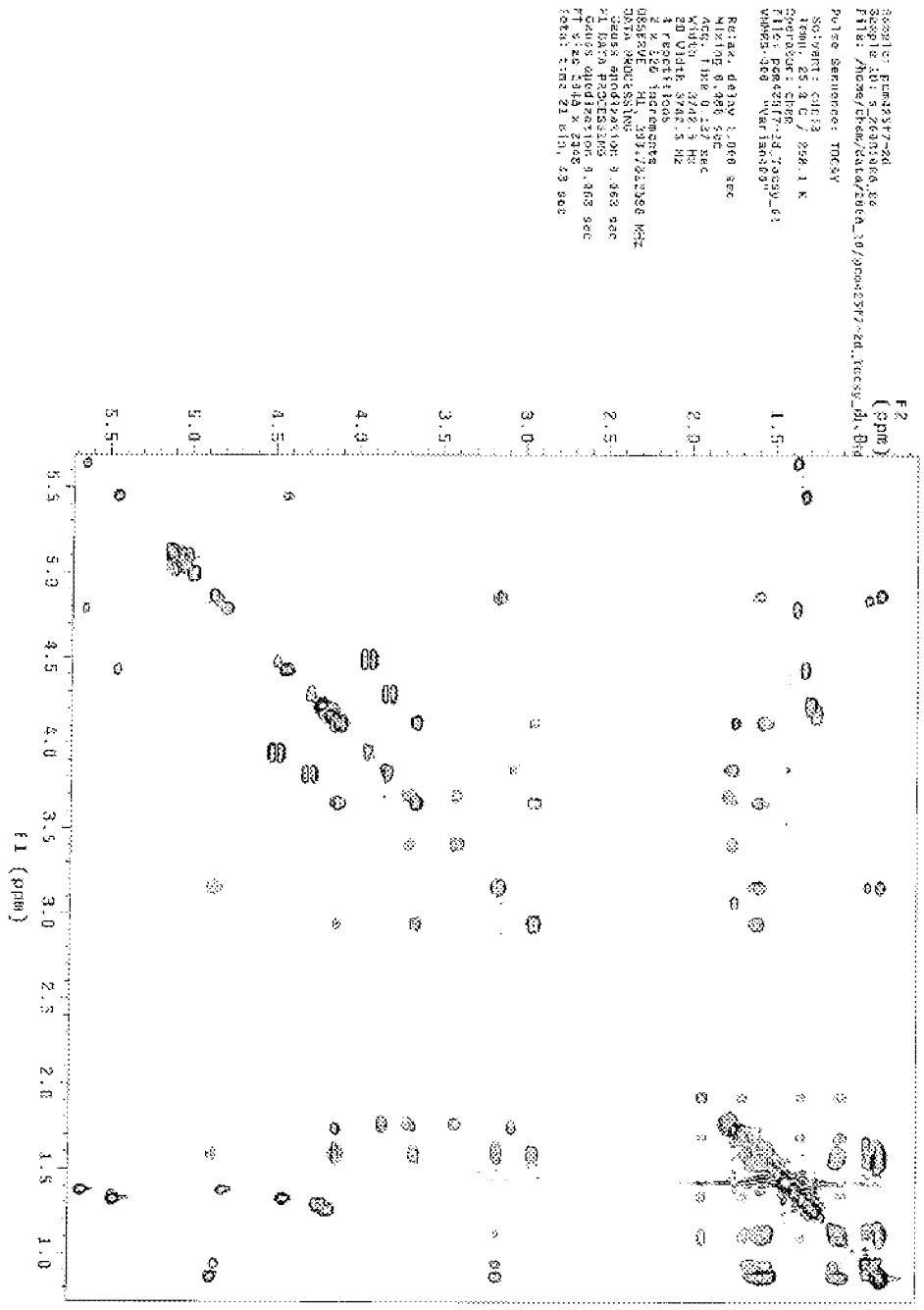

ppm
7
6
5
4
3
2

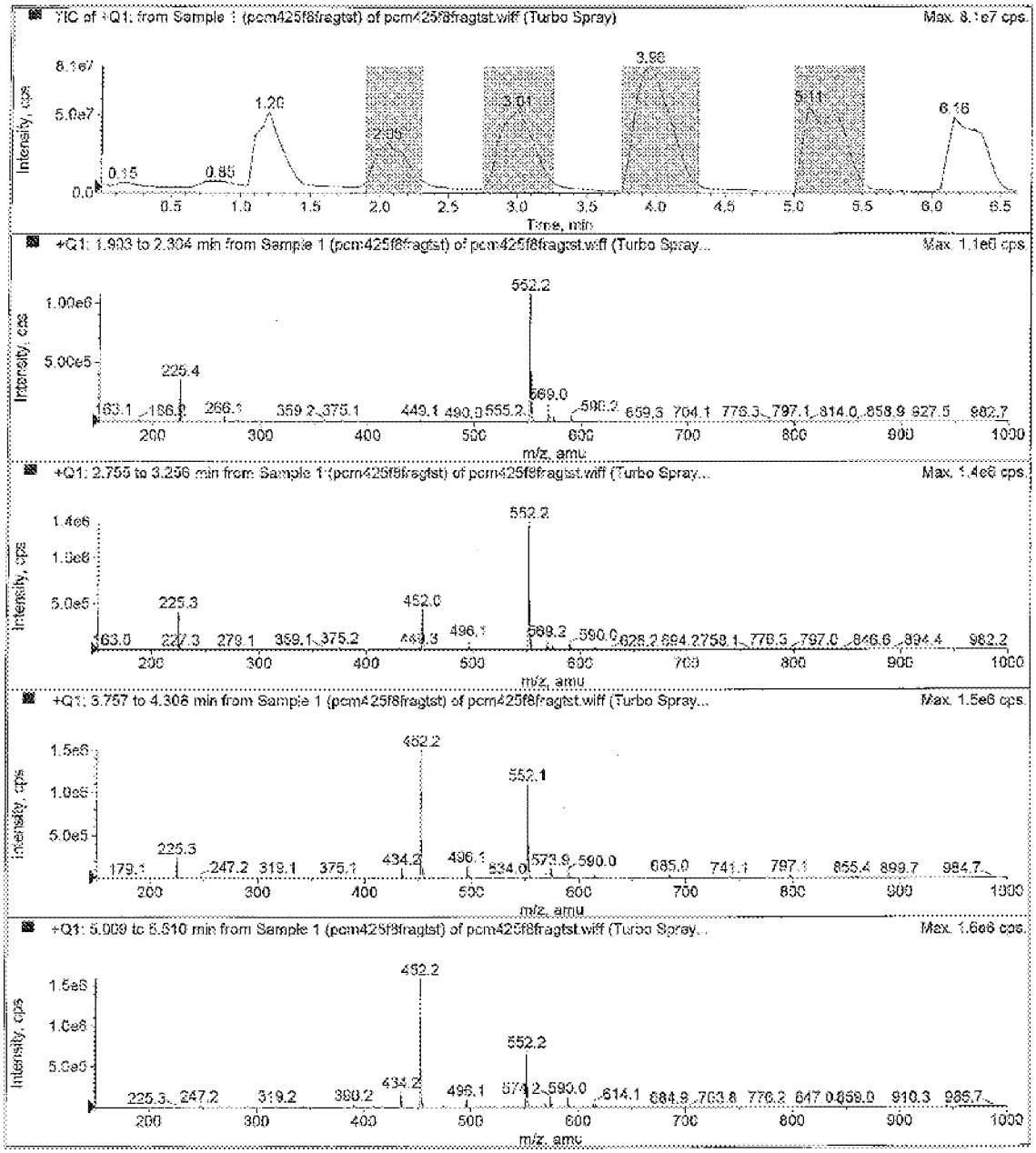

5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
ppm




ppm
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9

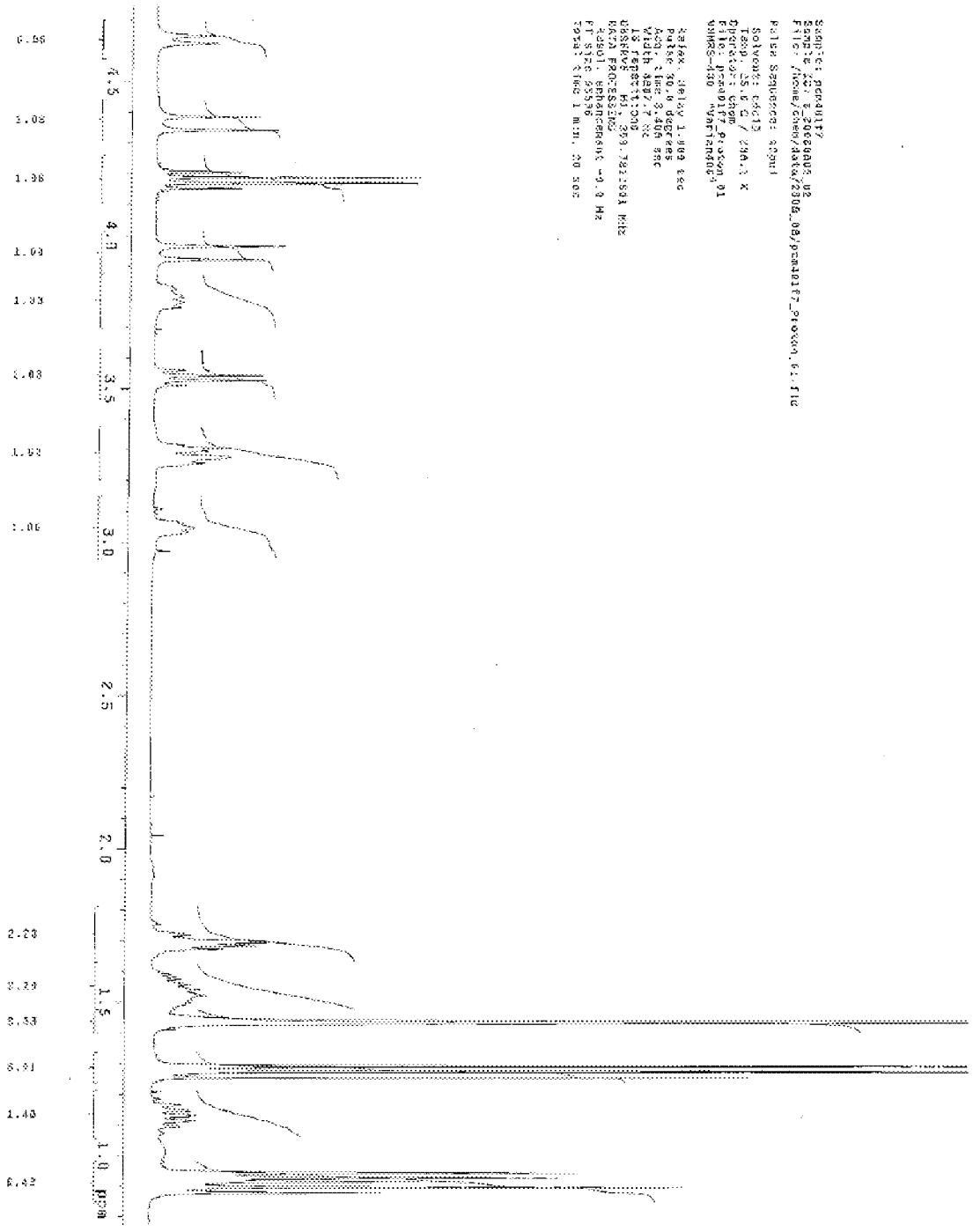

F2 (ppm)
F1 (ppm)

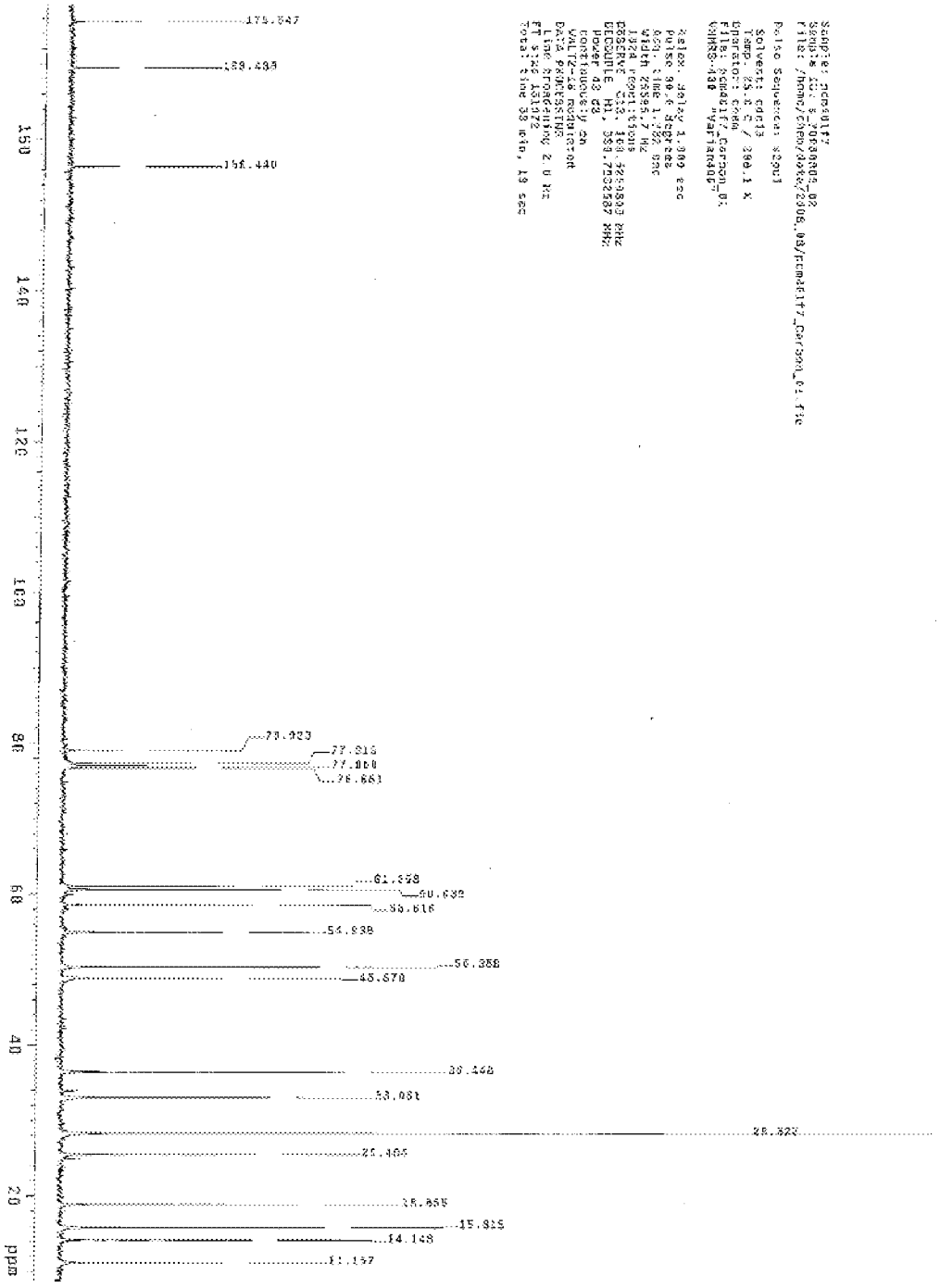

F2 (ppm)
F1 (ppm)

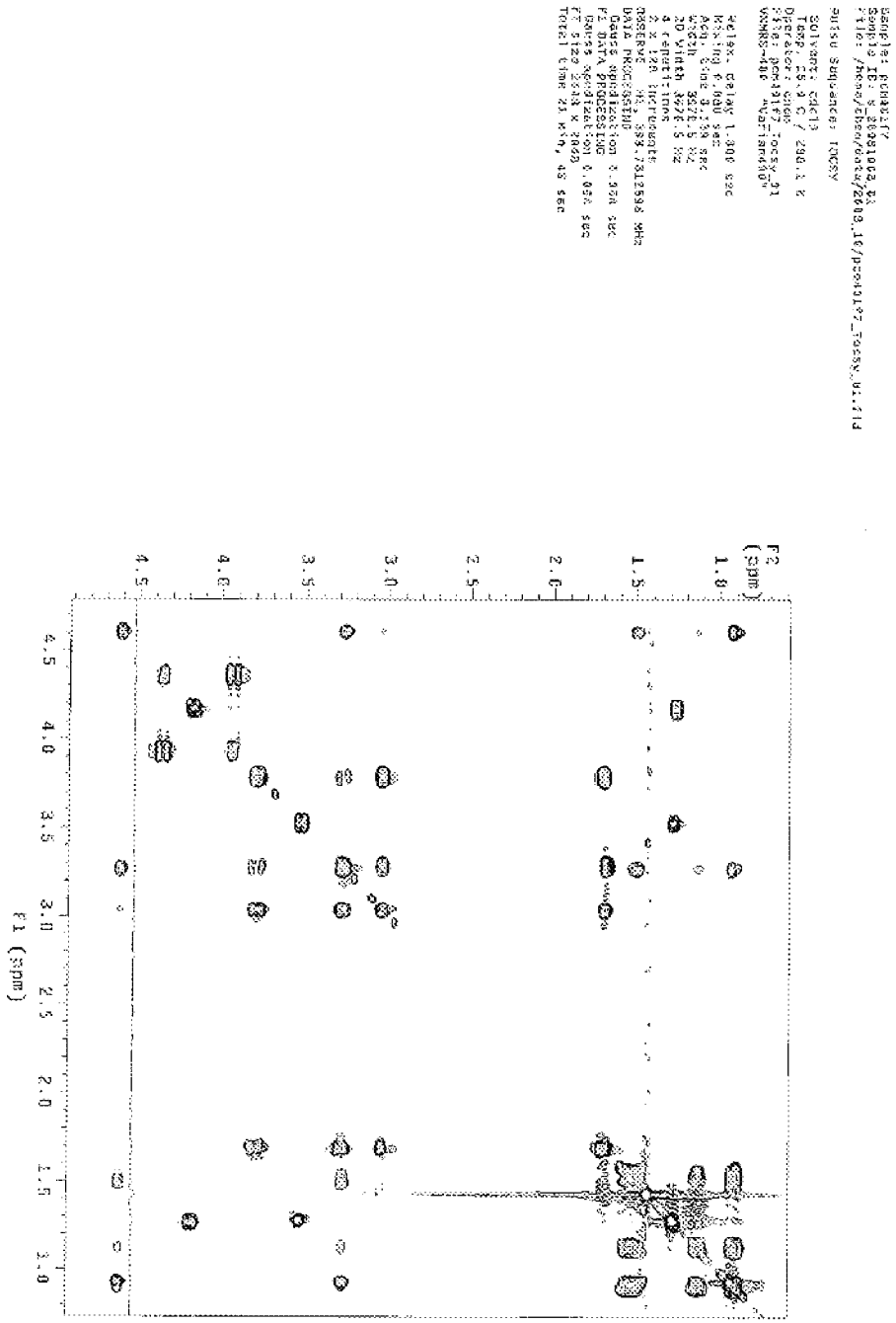

TIC of +Q1: from Sample 1 (pcm425f8fragtst) of pcm425f8fragtst.wiff (Turbo Spray)
Max. 8.1e7 cps.
Intensity, cps
Time, min
+Q1: 1.903 to 2.304 min from Sample 1 (pcm425f8fragtst) of pcm425f8fragtst.wiff (Turbo Spray...
Max. 1.1e6 cps.
552.2
225.4
669.0
m/z, amu
+Q1: 2.755 to 3.256 min from Sample 1 (pcm425f8fragtst) of pcm425f8fragtst.wiff (Turbo Spray...
Max. 1.4e6 cps.
552.2
225.3
452.0
m/z, amu
+Q1: 3.757 to 4.308 min from Sample 1 (pcm425f8fragtst) of pcm425f8fragtst.wiff (Turbo Spray...
Max. 1.5e6 cps.
452.2
552.1
225.3
m/z, amu
+Q1: 5.009 to 5.510 min from Sample 1 (pcm425f8fragtst) of pcm425f8fragtst.wiff (Turbo Spray...
Max. 1.6e6 cps.
452.2
552.2
225.3
m/z, amu

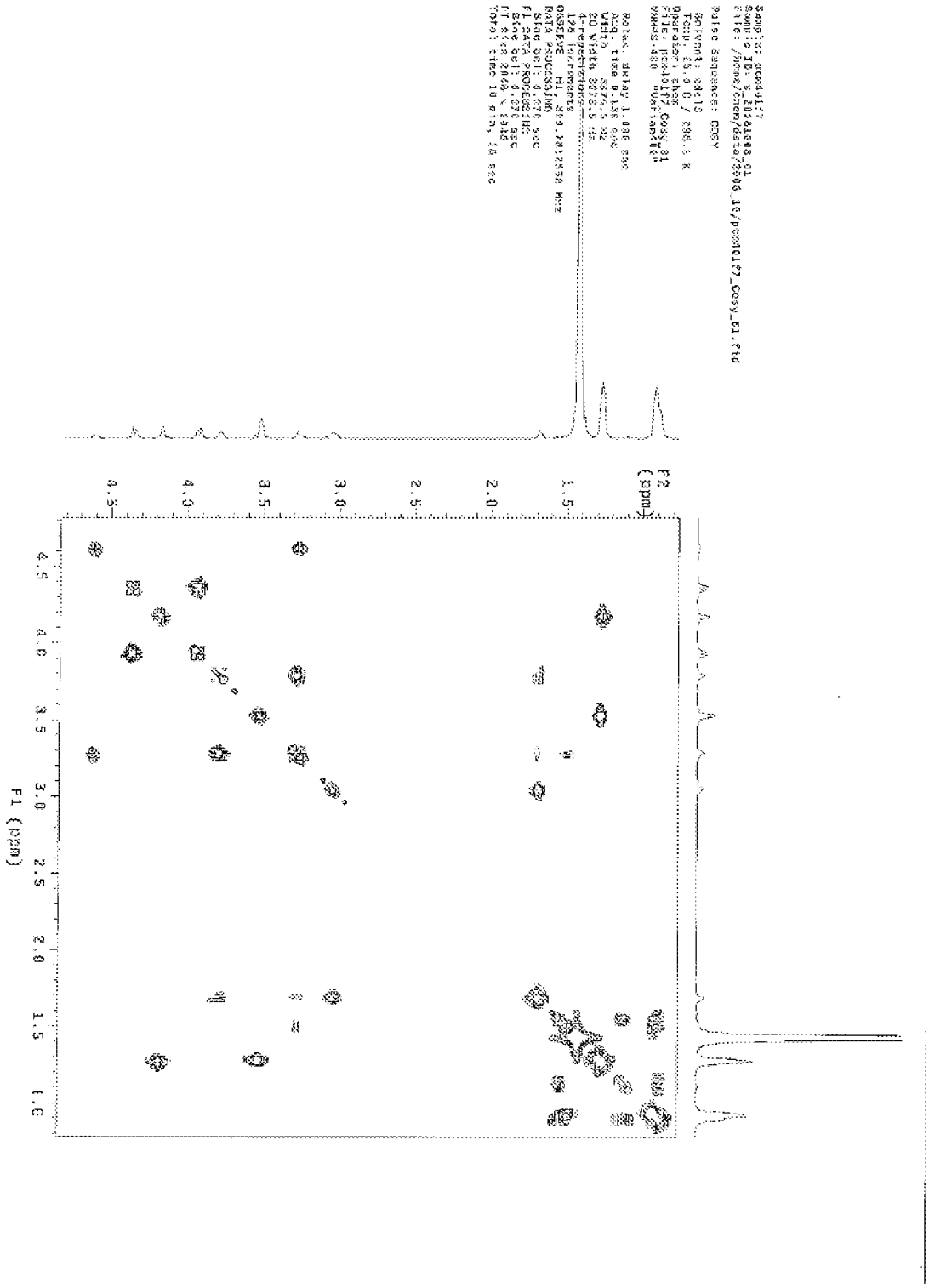
Appendix 6

Data obtained for Compound 14



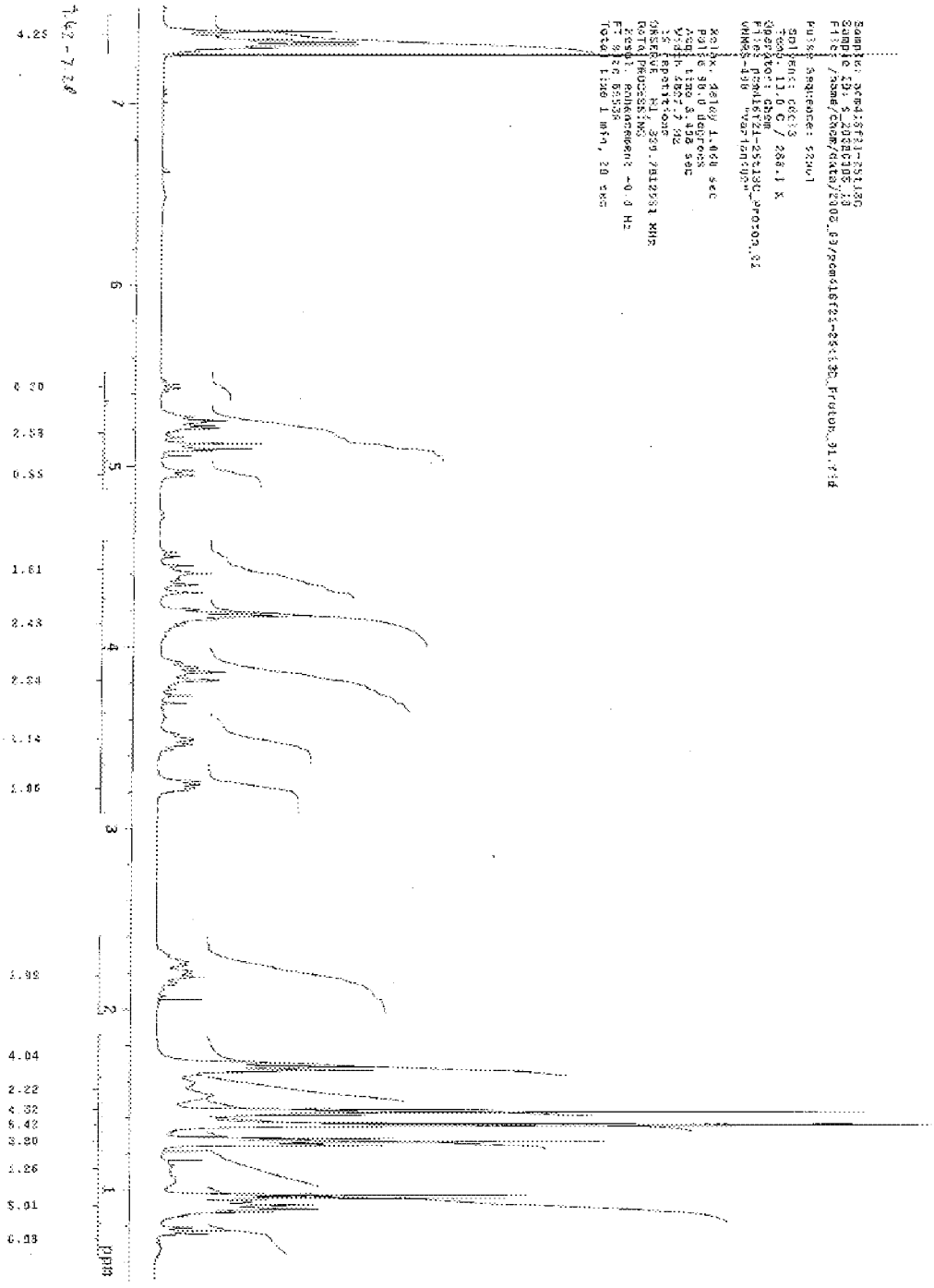

175.547
168.488
154.440
79.923
77.815
77.000
76.681
61.878
60.692
58.616
54.938
50.388
48.670
39.448
38.081
29.822
25.405
18.855
15.815
14.148
11.197
150
140
120
100
80
60
40
20
ppm

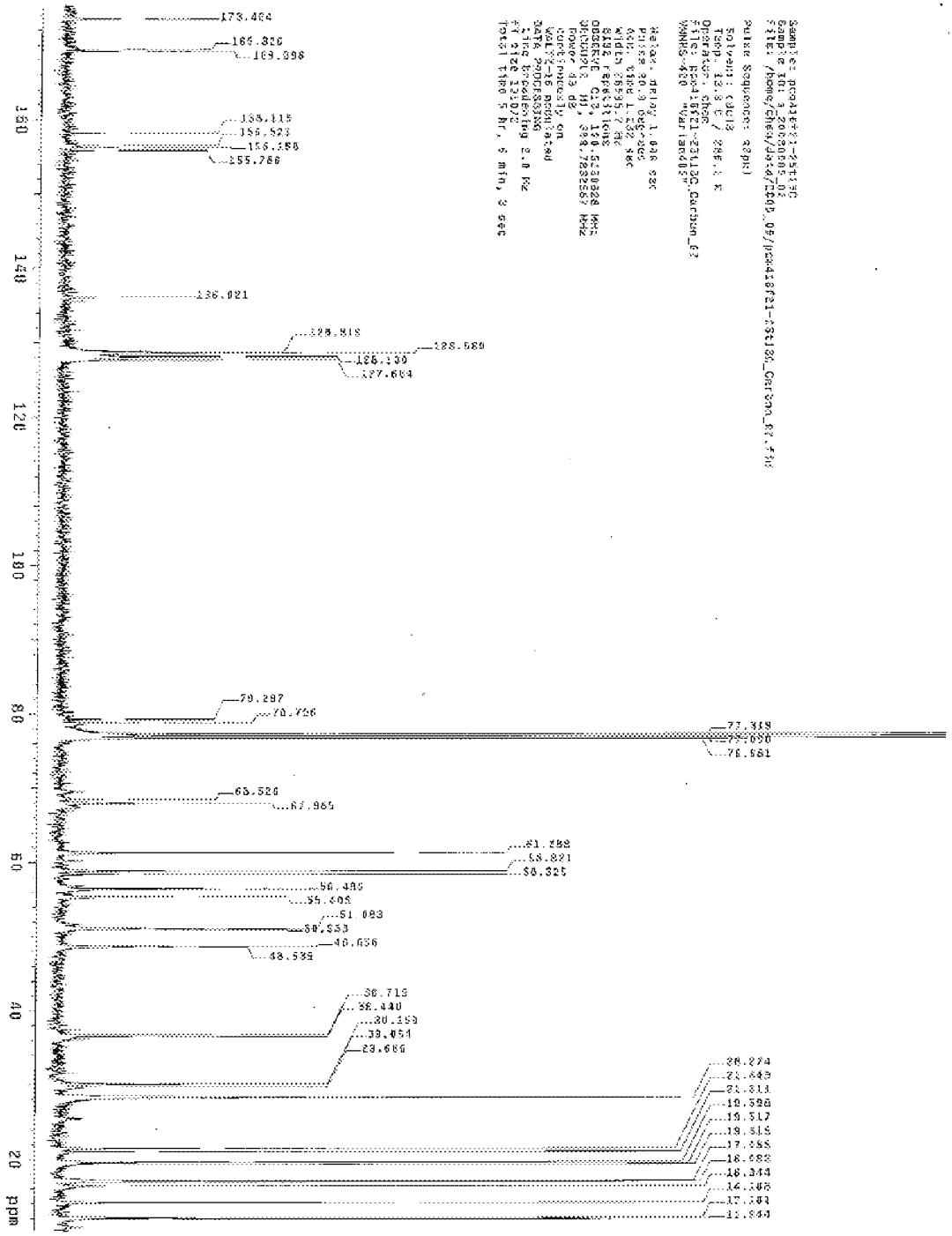

F2 (ppm)
F1 (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0

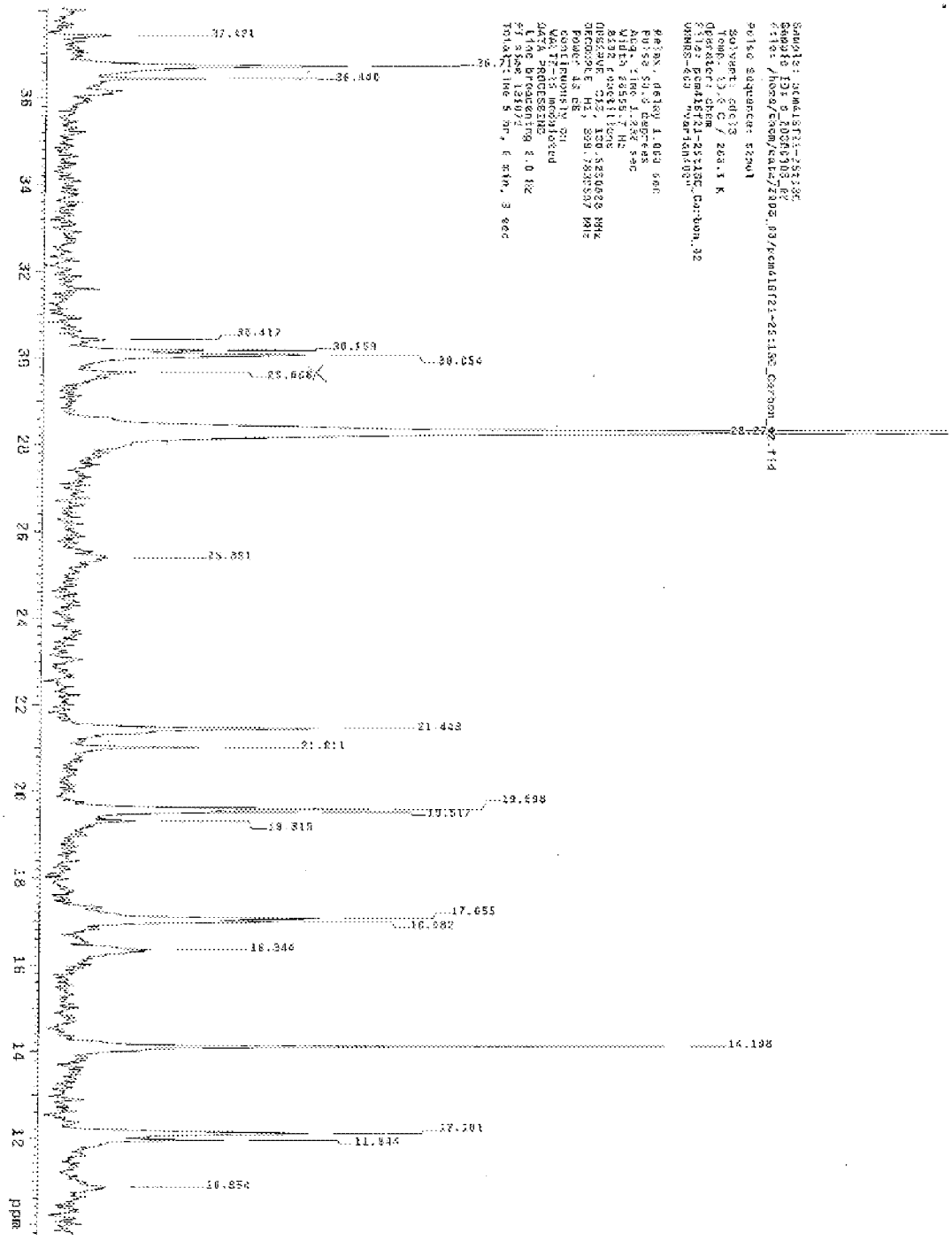

F2 (ppm)
F1 (ppm)

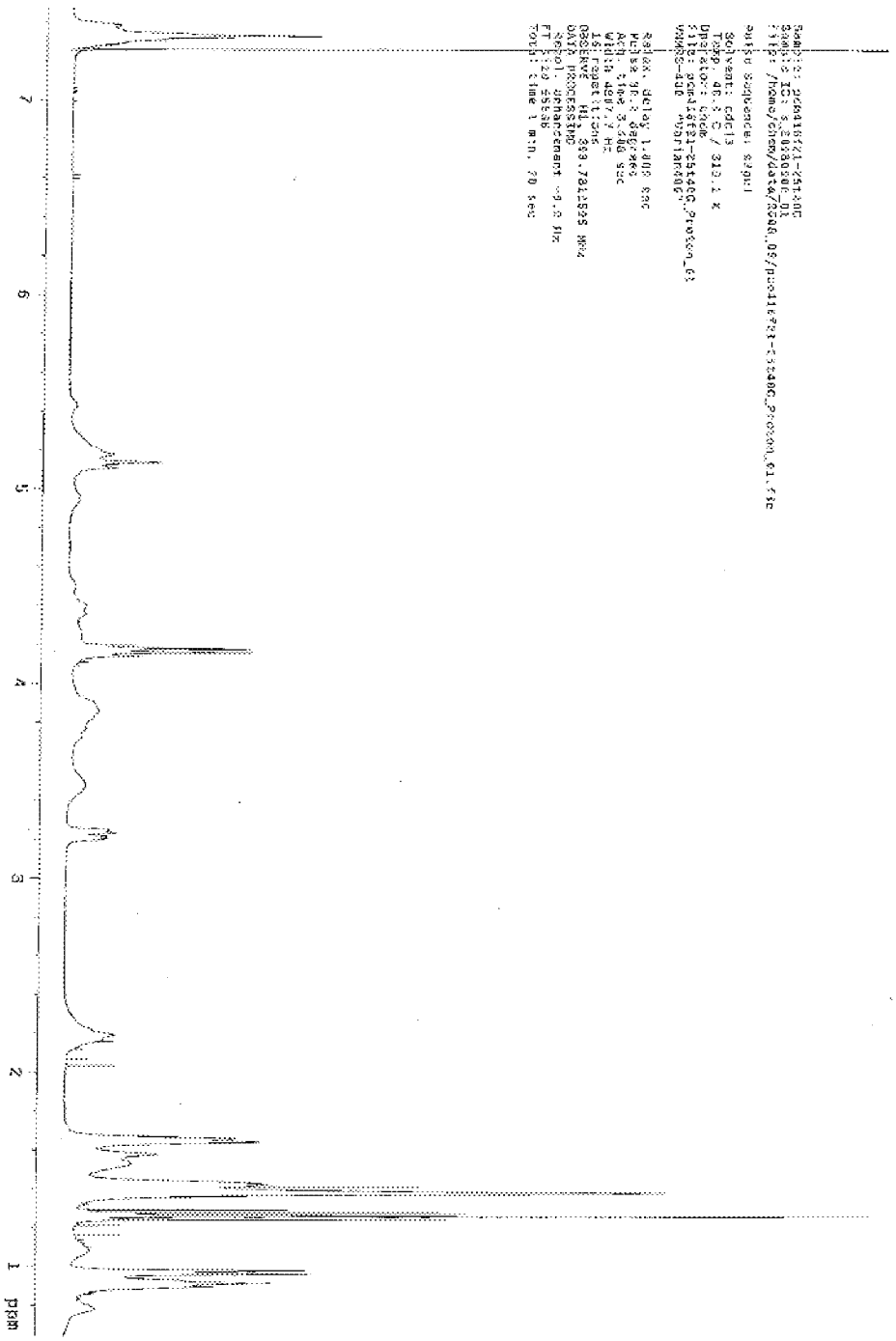
Appendix 7

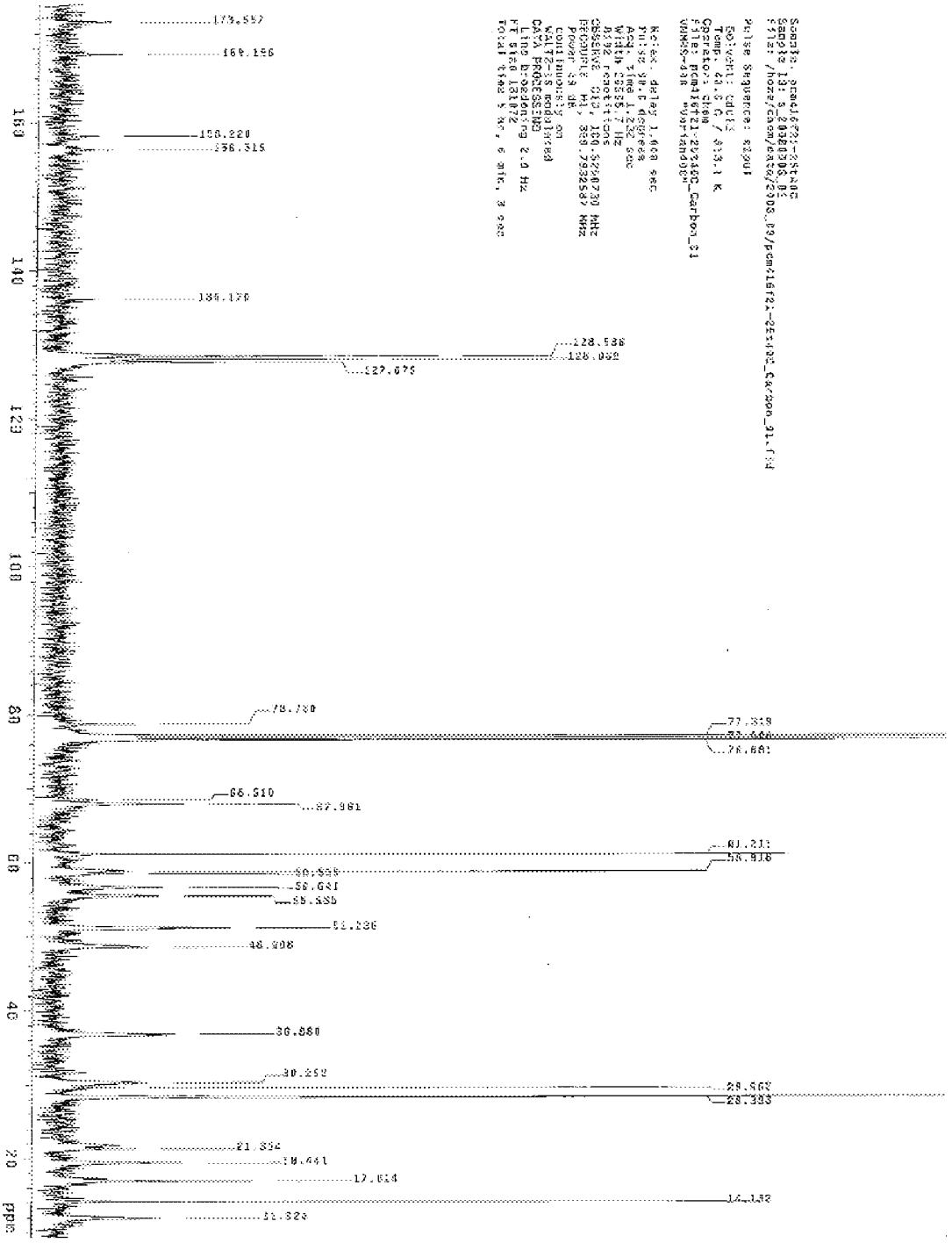
Data obtained for Compound 2

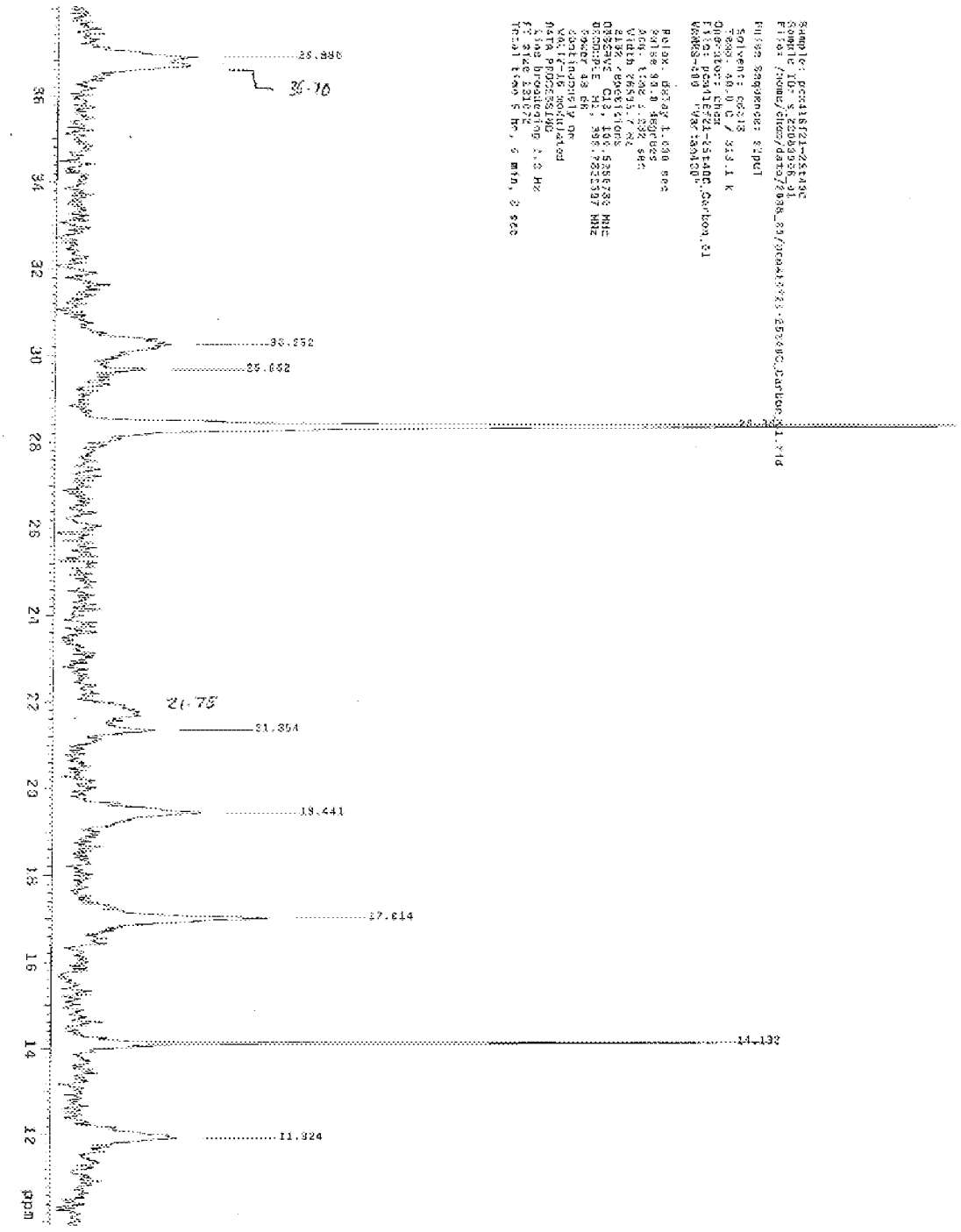

4.25
6.30
2.58
0.85
1.61
2.48
2.24
1.14
1.86
5.92
4.04
2.22
4.32
5.42
5.20
1.26
5.01
6.03
ppm

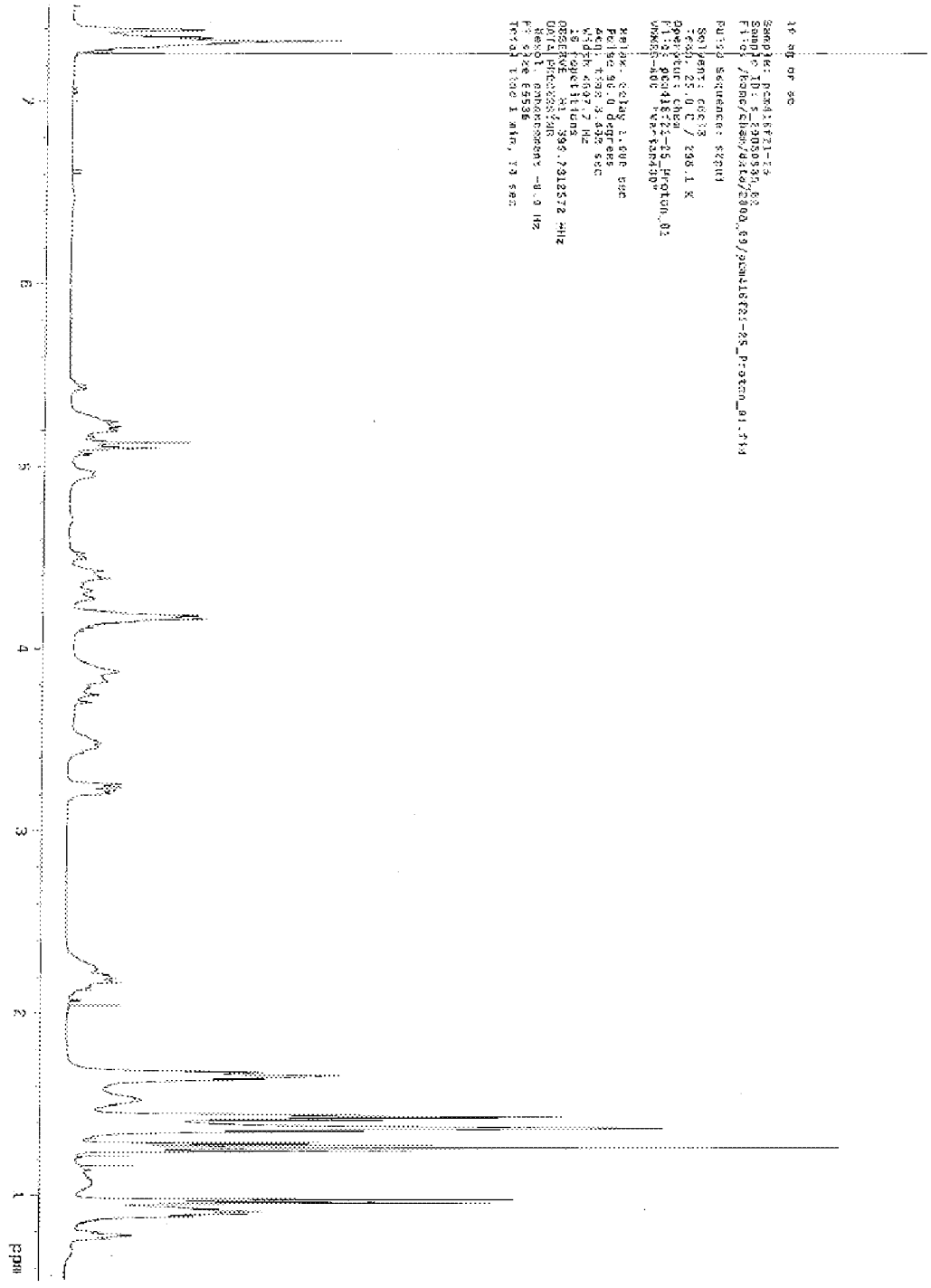

173.464
169.826
169.398
155.115
155.523
155.755
155.788
136.021
128.819
128.589
128.139
127.604
79.297
78.726
77.318
77.000
76.681
65.526
67.965
61.788
58.821
58.325
56.486
55.605
51.083
50.253
48.656
48.539
38.719
38.440
38.159
33.454
28.686
28.274
21.649
21.311
19.598
19.517
19.515
17.555
16.683
16.344
14.186
13.181
11.844
160
140
120
100
80
60
40
20
ppm

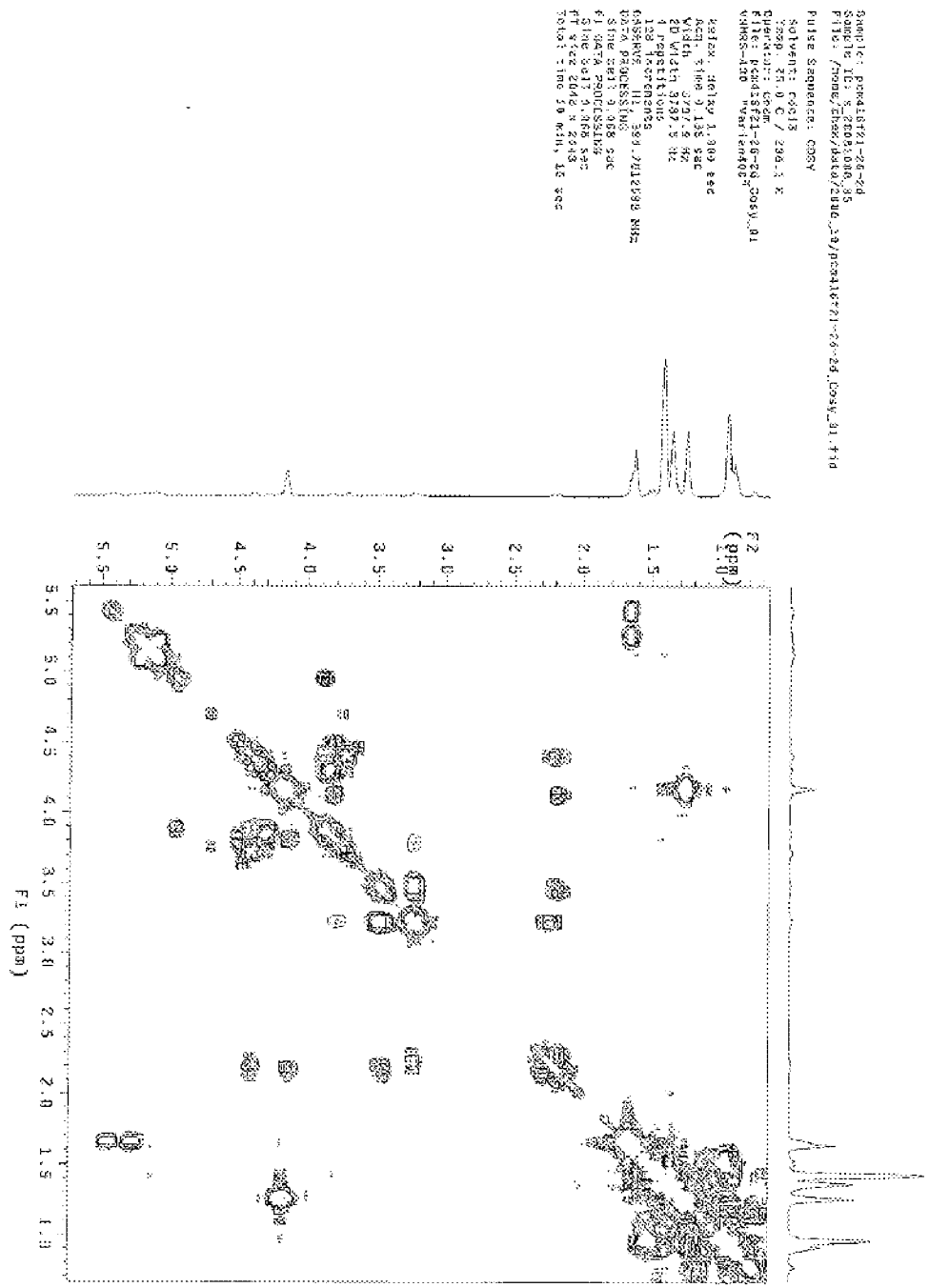

37.691
36.
36.480
38.417
38.159
38.054
28.276
25.881
21.433
21.211
19.698
19.517
18.315
17.655
16.982
16.346
14.198
12.701
11.944
10.856
ppm

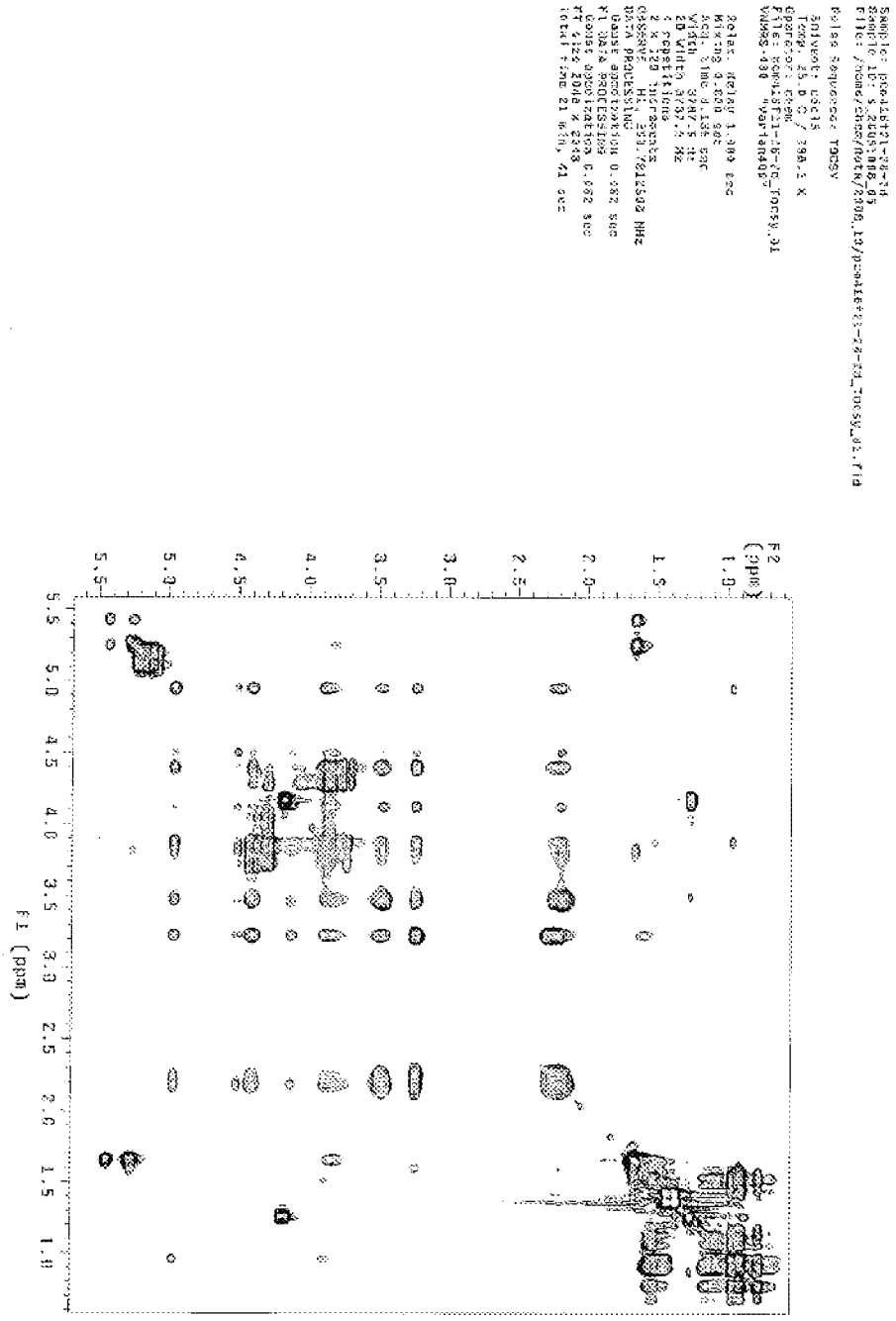

ppm
1
2
3
4
5
6
7

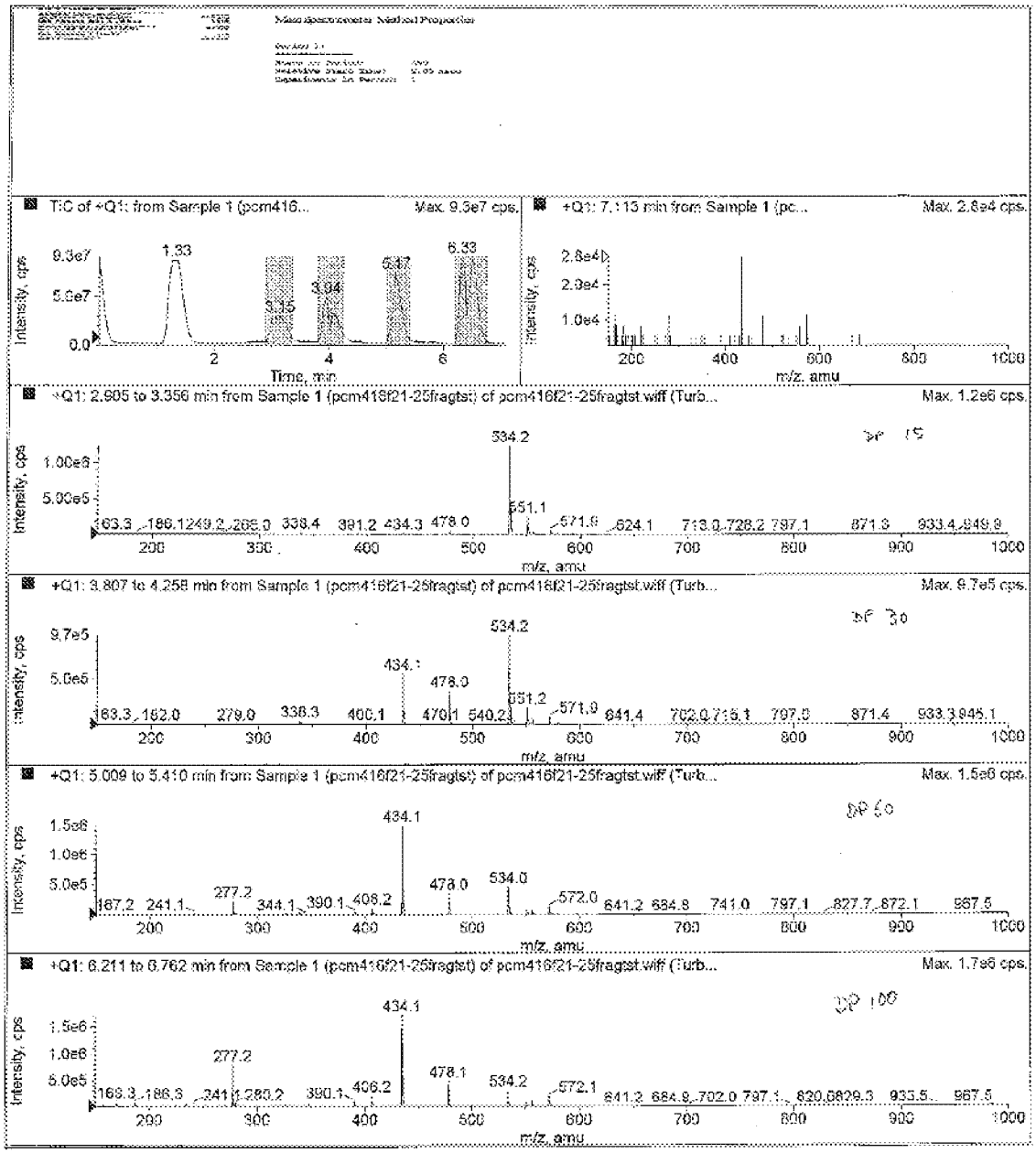

173.557
169.196
158.220
156.515
138.179
128.538
128.052
127.675
78.730
77.319
77.000
76.681
66.510
57.981
60.211
58.918
50.535
50.641
48.908
36.880
30.252
28.662
28.383
21.852
19.441
17.614
14.132
11.324
ppm

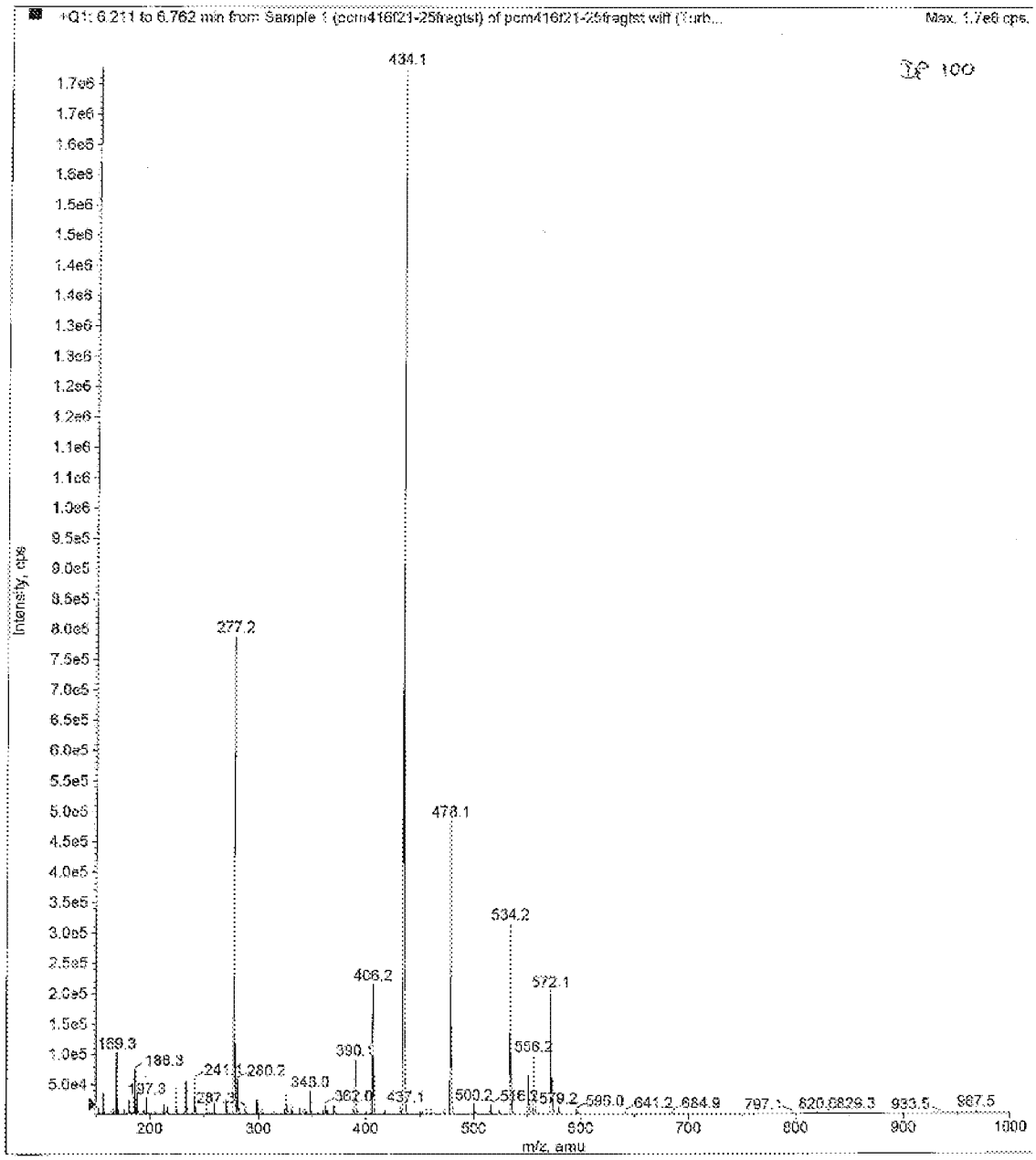

25.886
36.10
30.852
29.692
21.75
21.364
18.441
17.614
14.132
11.924
ppm


ppm
1
2
3
4
5
6
7


F2 (ppm)
F1 (ppm)

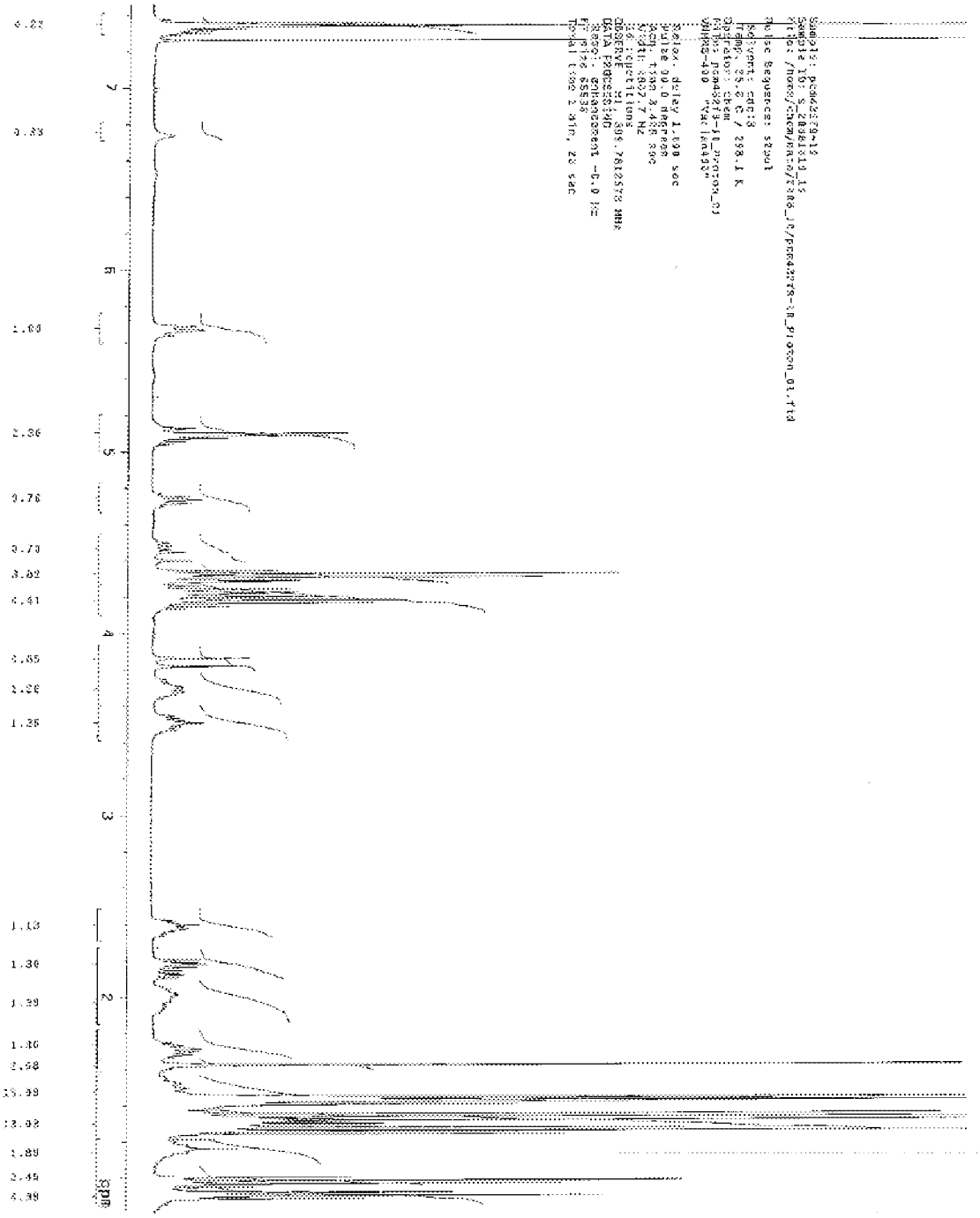

F2 (ppm)
F1 (ppm)

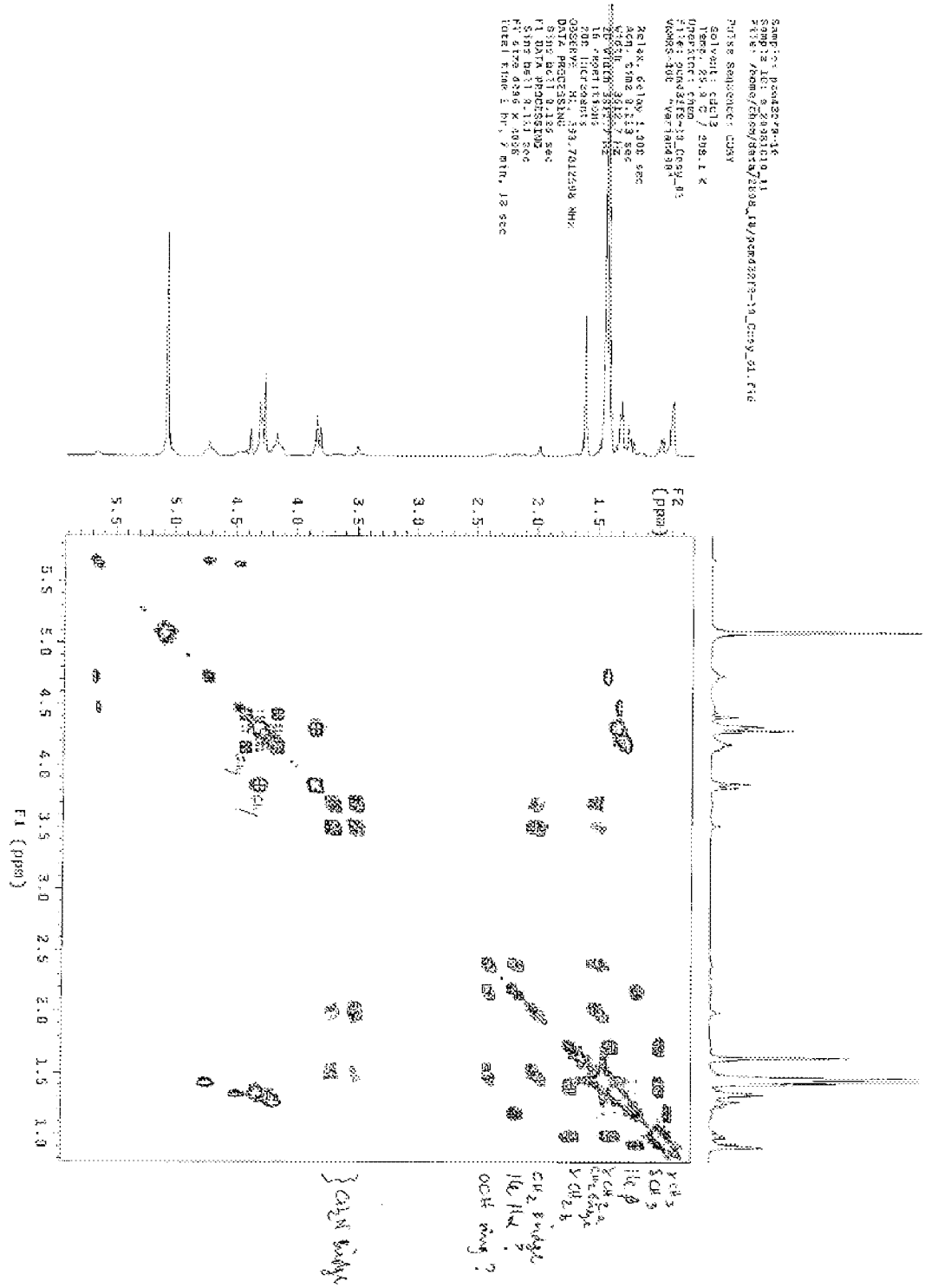

TIC of +Q1: from Sample 1 (pcm416...
Max. 9.3e7 cps.
Intensity, cps
Time, min
+Q1: 7.113 min from Sample 1 (pc...
Max. 2.8e4 cps.
m/z, amu
+Q1: 2.905 to 3.356 min from Sample 1 (pcm416f21-25fragtst) of pcm416f21-25fragtst.wiff (Turb...
Max. 1.2e6 cps.
534.2
DP 15
+Q1: 3.807 to 4.258 min from Sample 1 (pcm416f21-25fragtst) of pcm416f21-25fragtst.wiff (Turb...
Max. 9.7e5 cps.
534.2
434.1
478.0
DP 30
+Q1: 5.009 to 5.410 min from Sample 1 (pcm416f21-25fragtst) of pcm416f21-25fragtst.wiff (Turb...
Max. 1.5e6 cps.
434.1
478.0
534.0
DP 50
+Q1: 6.211 to 6.762 min from Sample 1 (pcm416f21-25fragtst) of pcm416f21-25fragtst.wiff (Turb...
Max. 1.7e6 cps.
434.1
277.2
478.1
534.2
DP 100

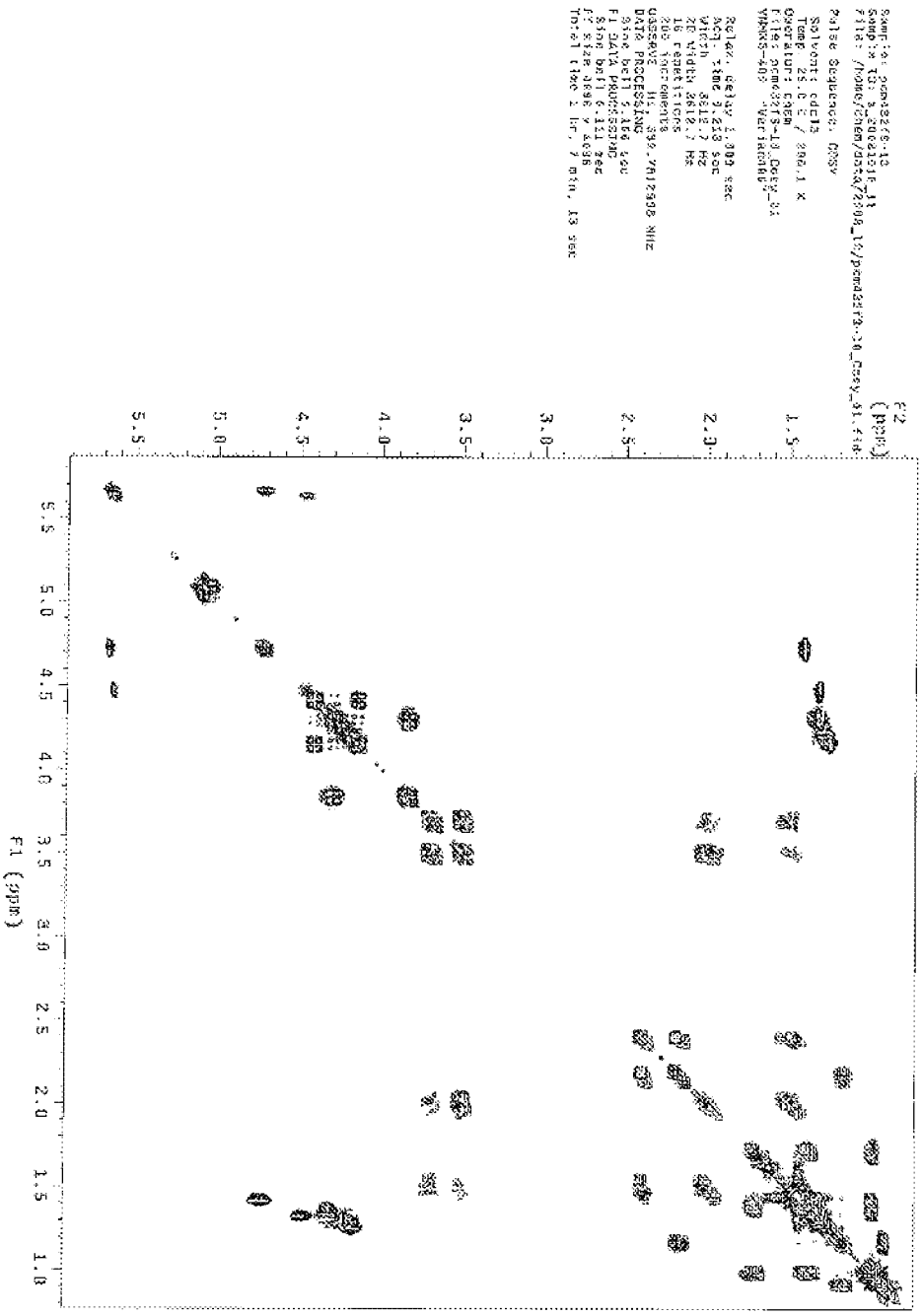

+Q1: 6.211 to 6.762 min from Sample 1 (pcm416f21-25fragtst) of pcm416f21-25fragtst.wiff (Turb...
Max. 1.7e6 cps.
434.1
277.2
478.1
534.2
406.2
572.1
556.2
169.3
188.3
390.3
280.2
348.0
Intensity, cps
m/z, amu

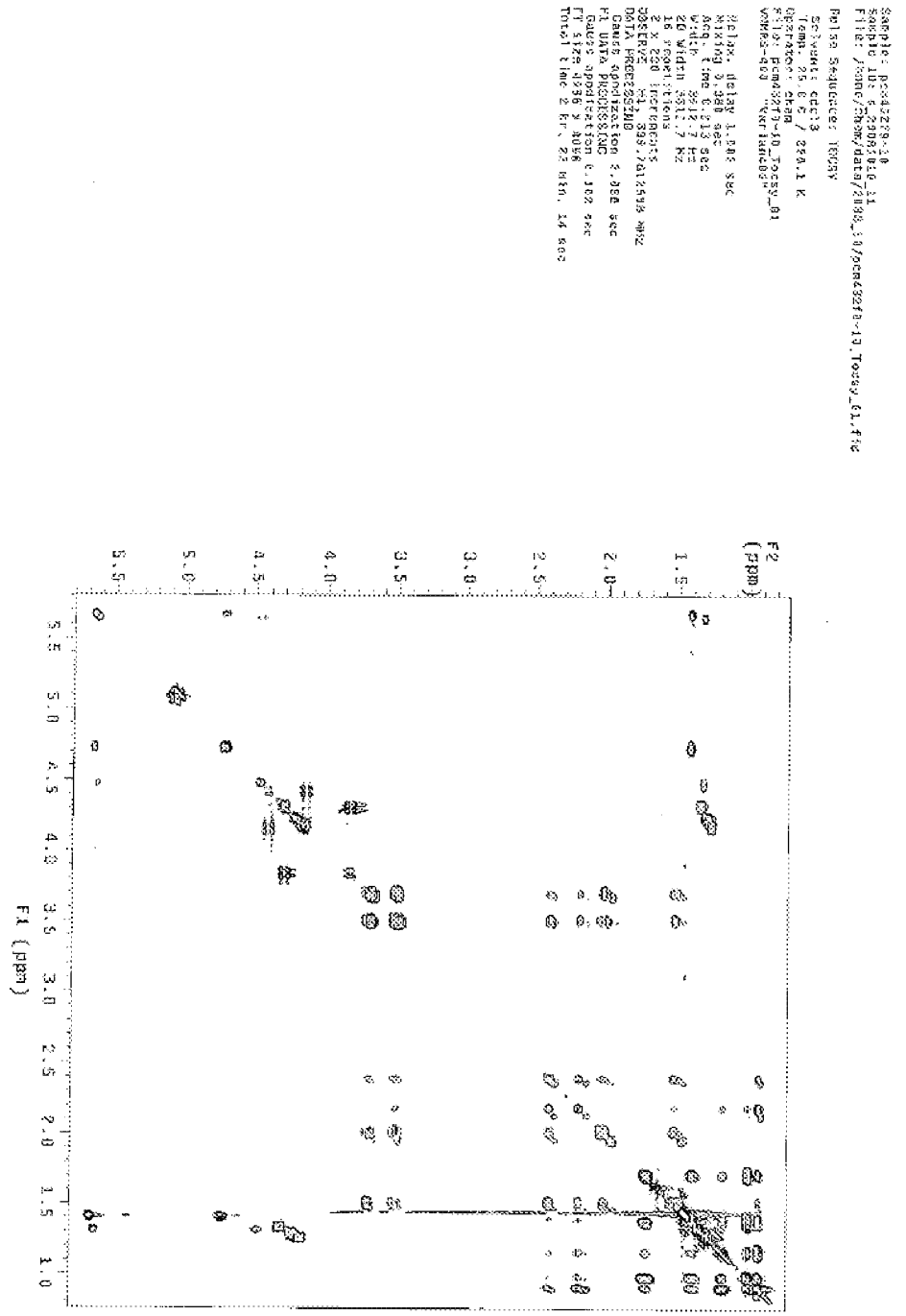
Appendix 8

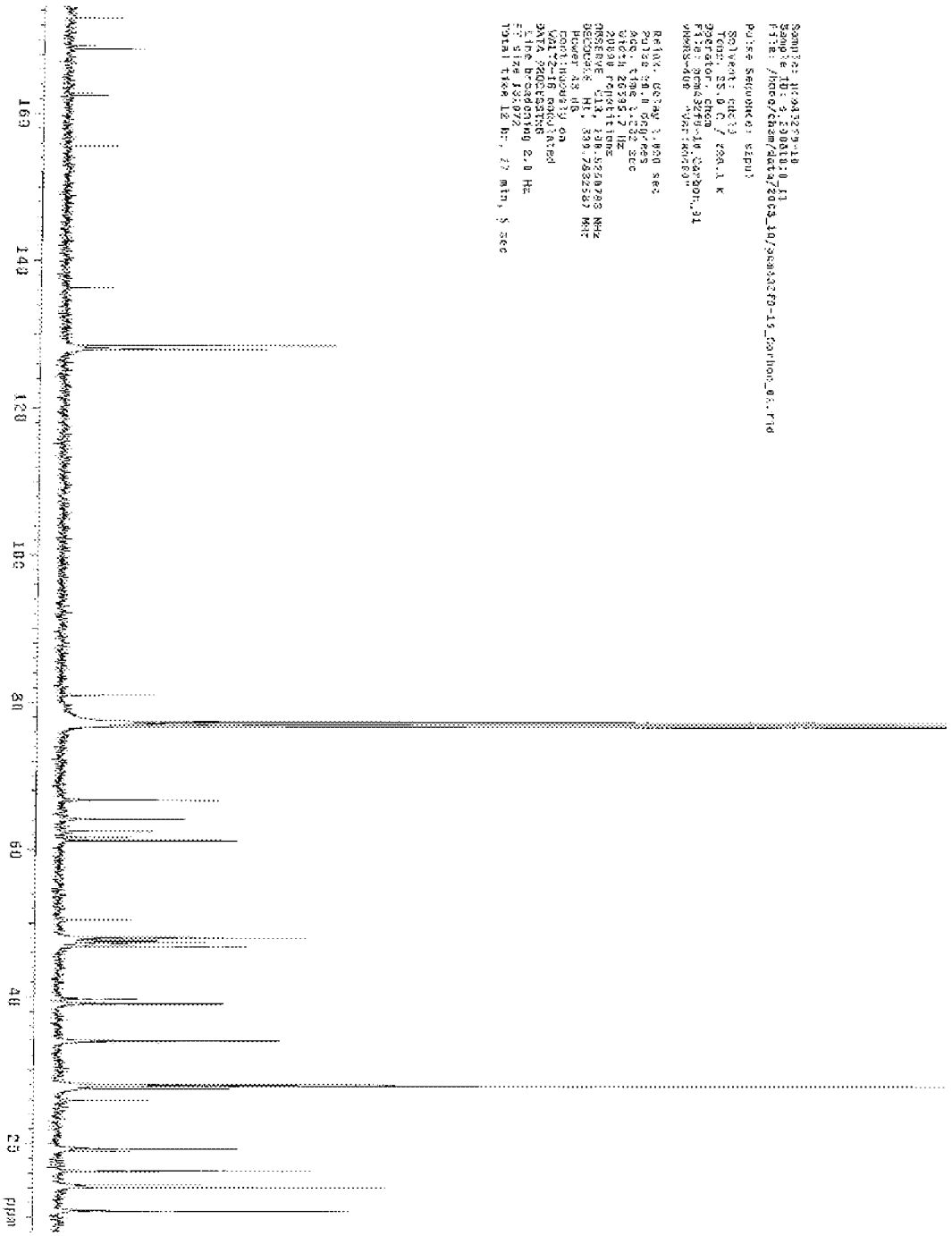
Data obtained for Compounds 3a

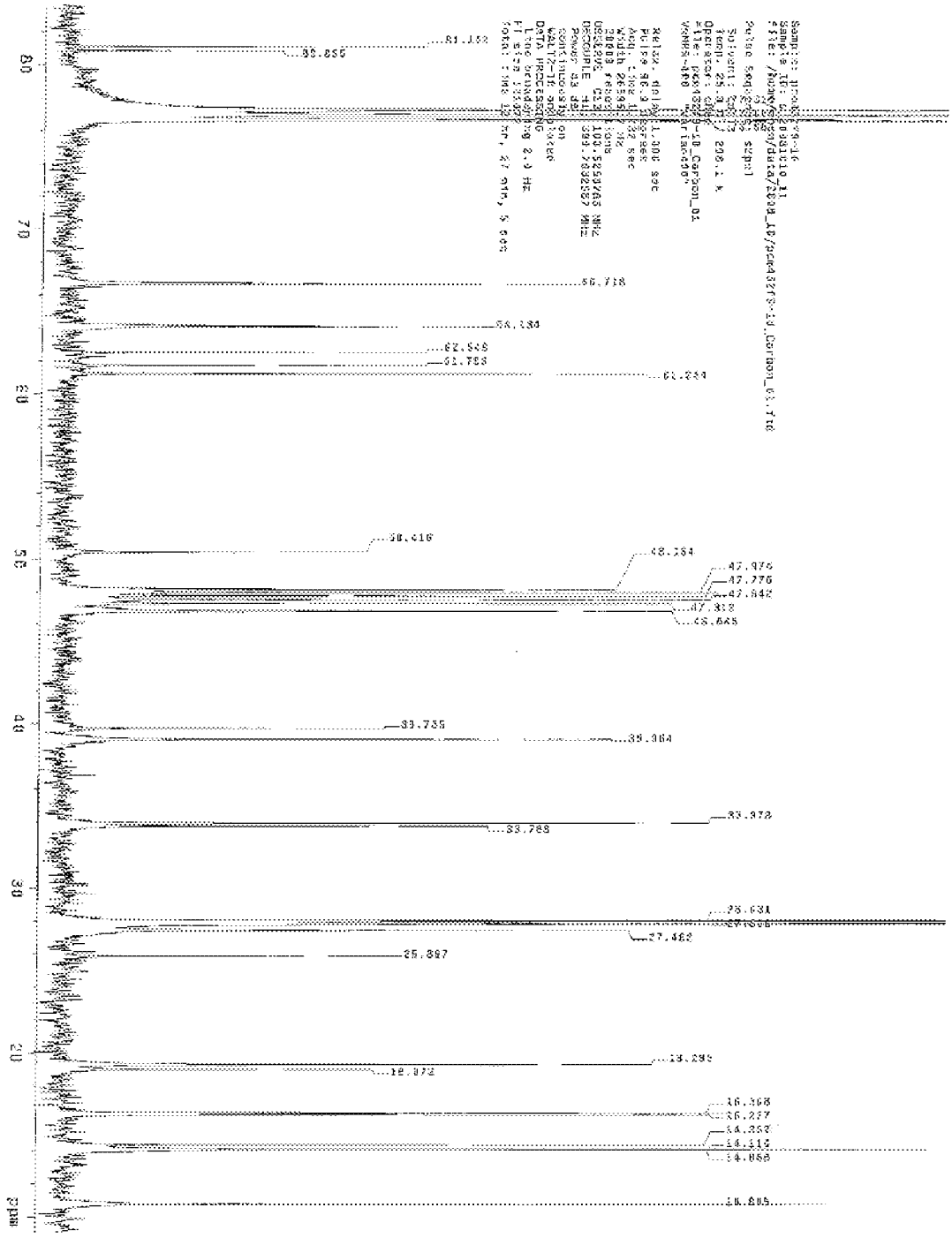


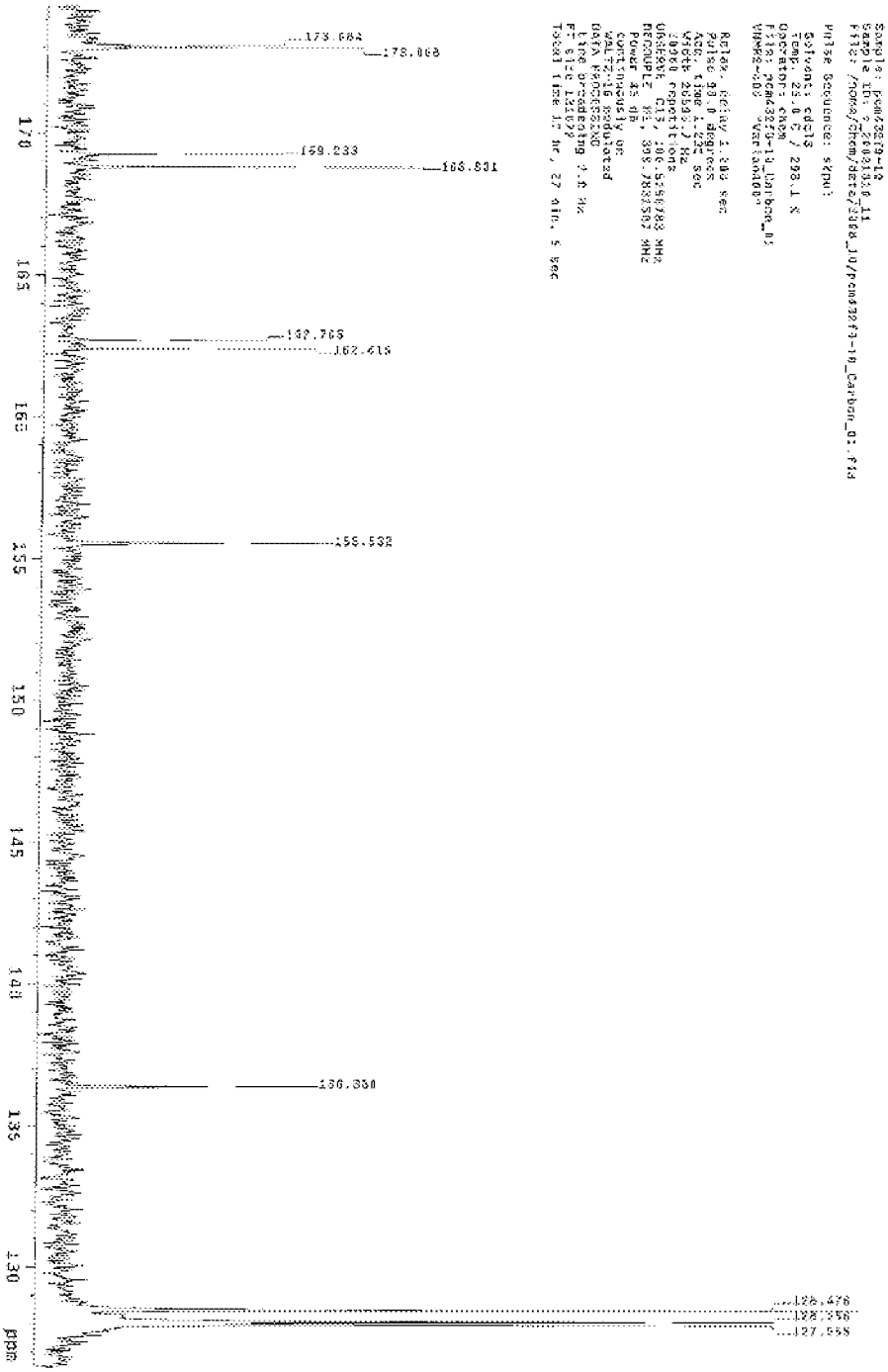


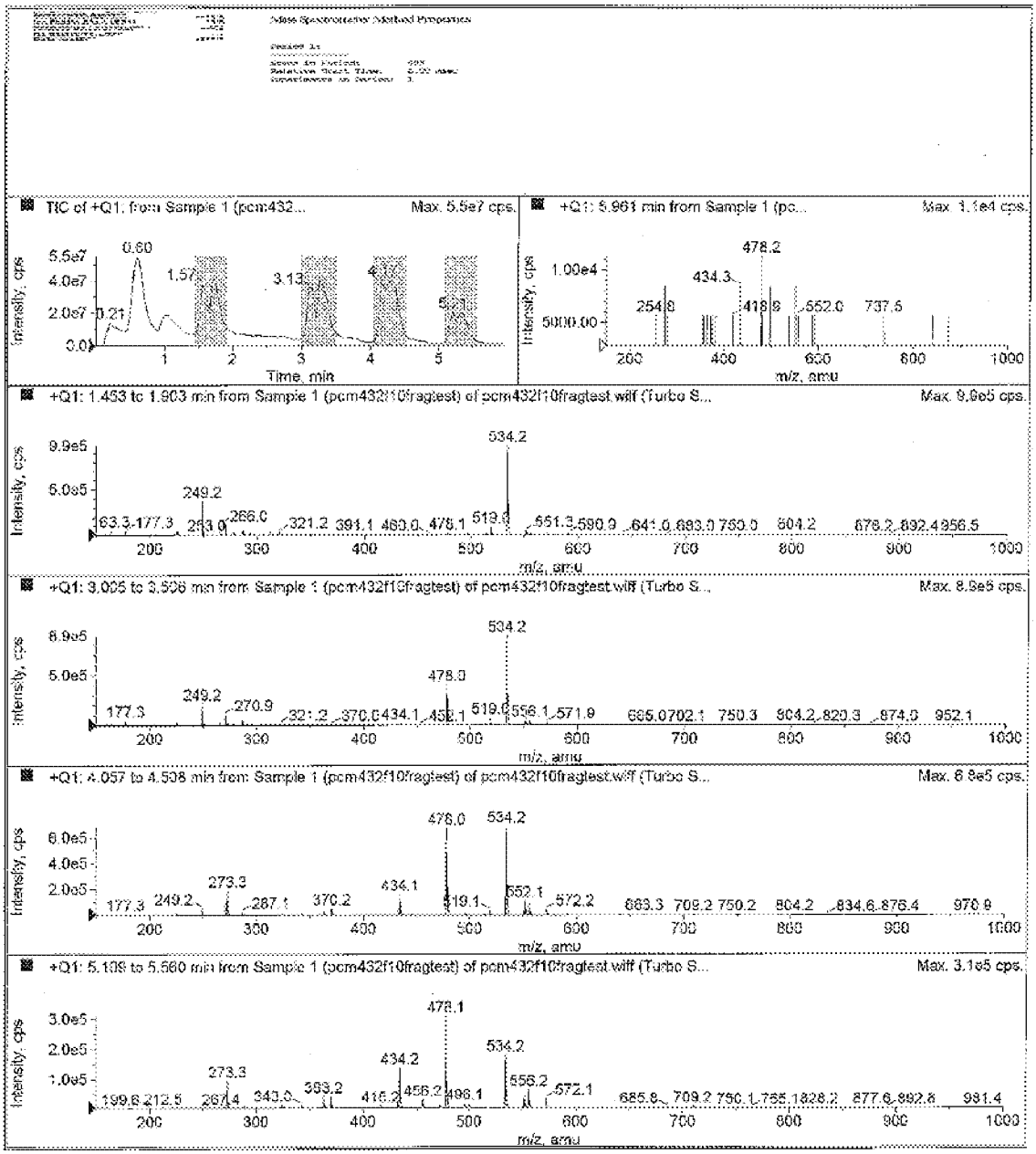

F2 (ppm)
F1 (ppm)

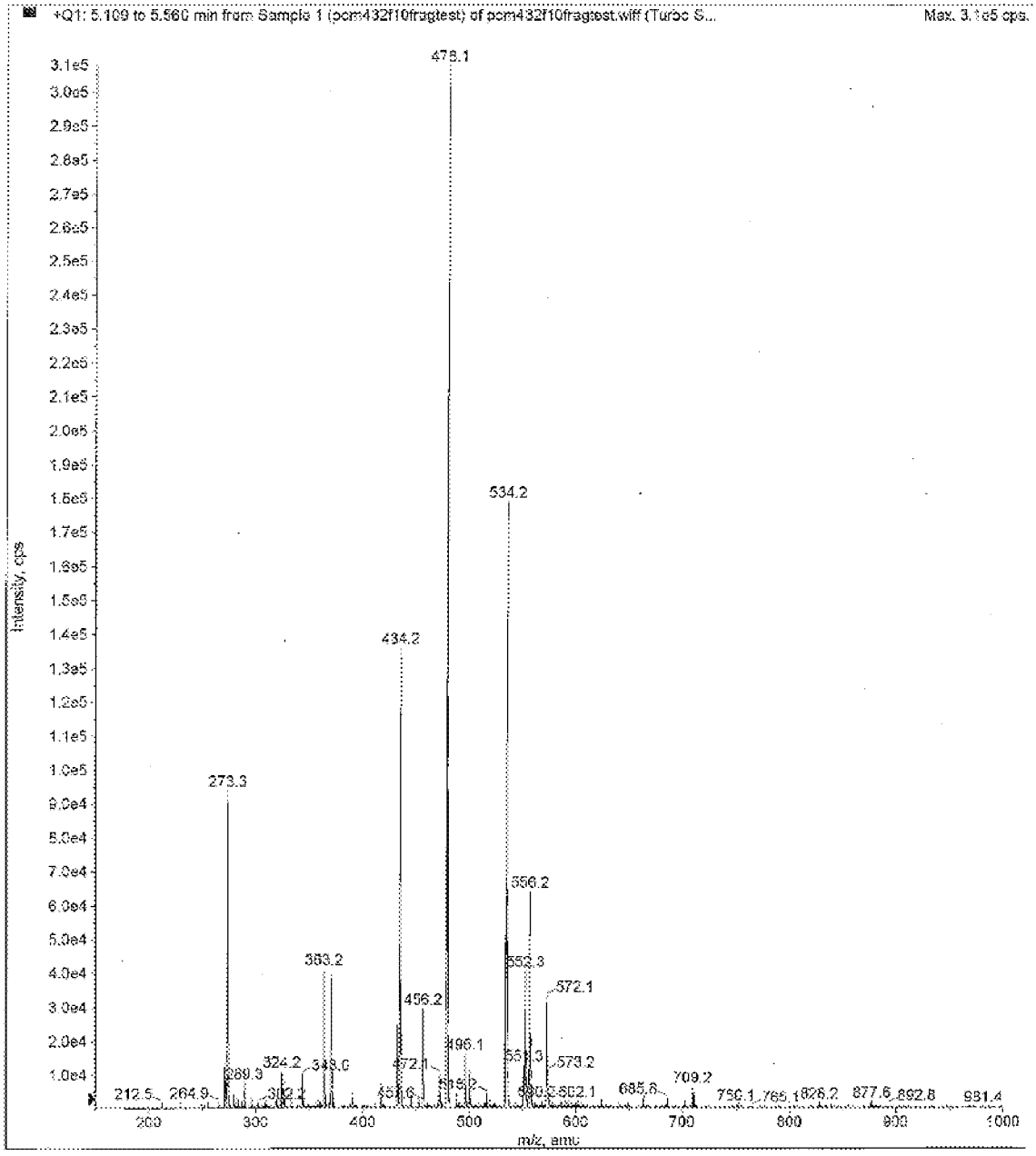

F2 (ppm)
F1 (ppm)

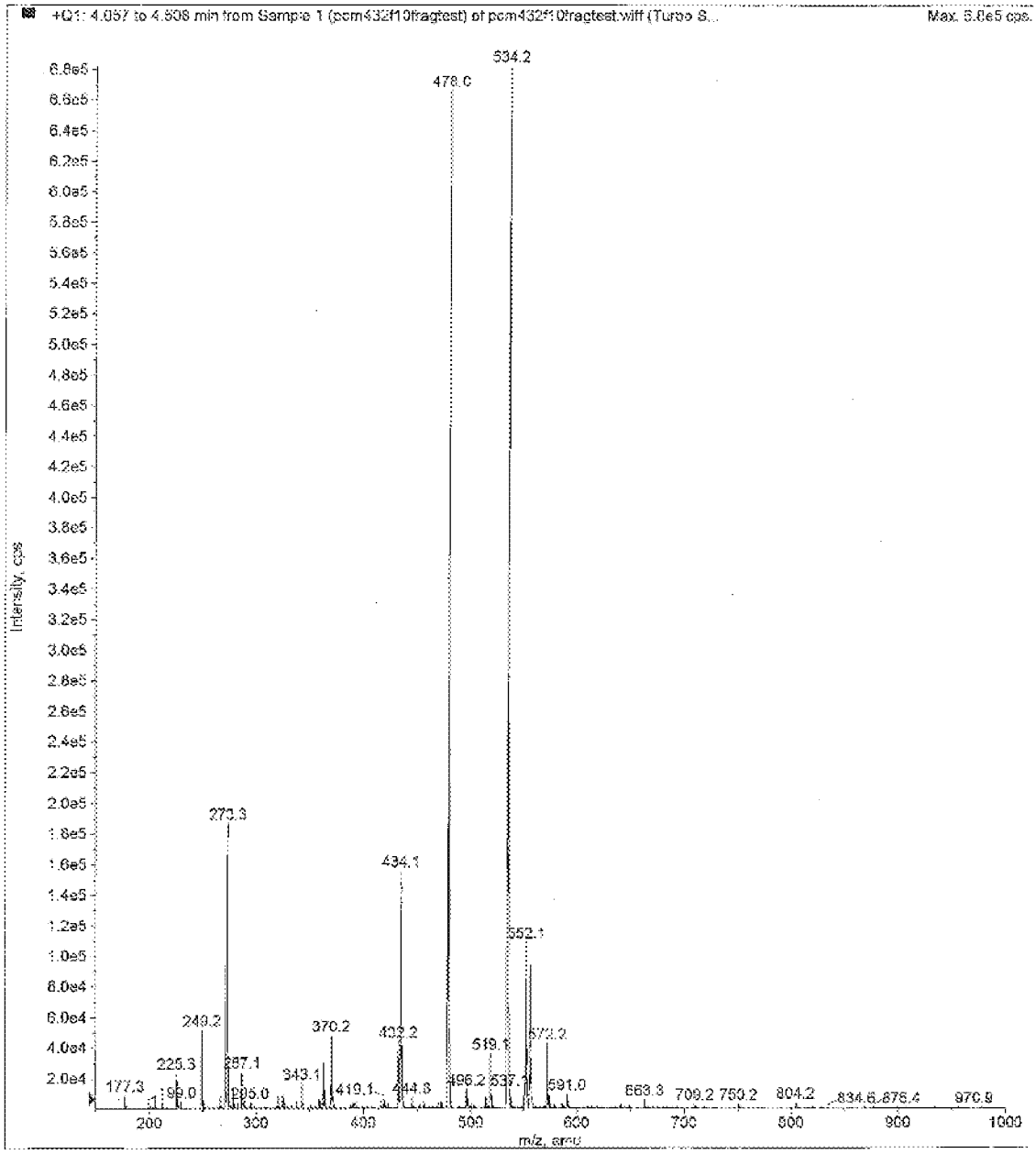

160
140
120
100
80
60
40
20
ppm


80
70
60
50
40
30
20
ppm
61.152
60.855
56.718
56.284
52.548
51.783
51.284
48.416
48.284
47.976
47.776
47.642
47.312
46.845
39.755
39.364
33.972
33.788
25.631
27.462
25.897
18.299
18.072
16.368
16.257
14.352
14.112
14.058
10.805


173.682
173.068
169.233
166.831
162.709
162.415
155.532
136.330
128.476
128.356
127.558
170
165
160
155
150
145
140
135
130
ppm

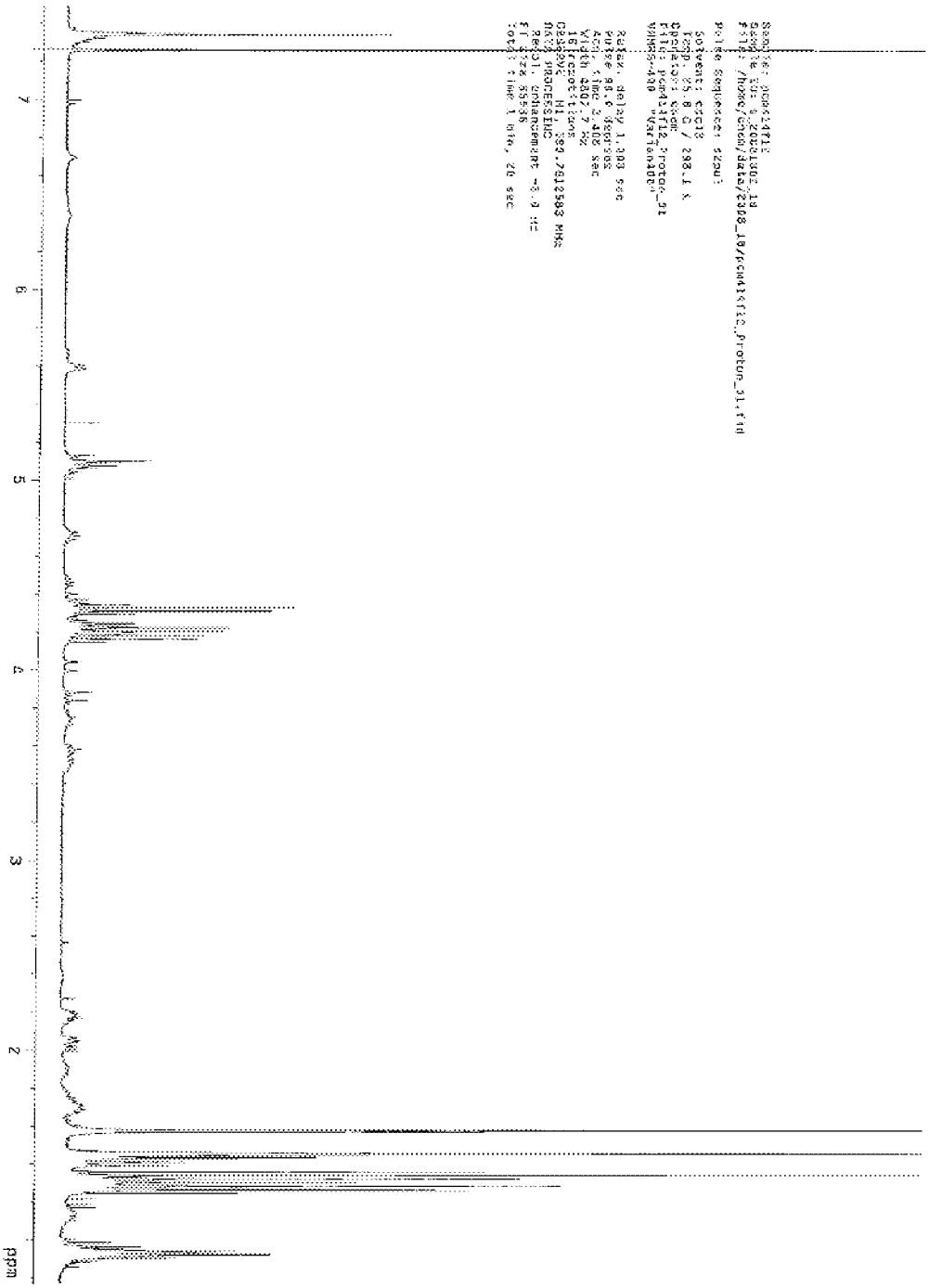

TIC of +Q1, from Sample 1 (pcm432...
Max. 5.5e7 cps.
Intensity, cps
Time, min
+Q1: 5.961 min from Sample 1 (pc...
Max. 1.1e4 cps.
m/z, amu
+Q1: 1.453 to 1.903 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 9.9e5 cps.
+Q1: 3.005 to 3.506 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 8.9e5 cps.
+Q1: 4.057 to 4.508 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 6.8e5 cps.
+Q1: 5.109 to 5.560 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 3.1e5 cps.

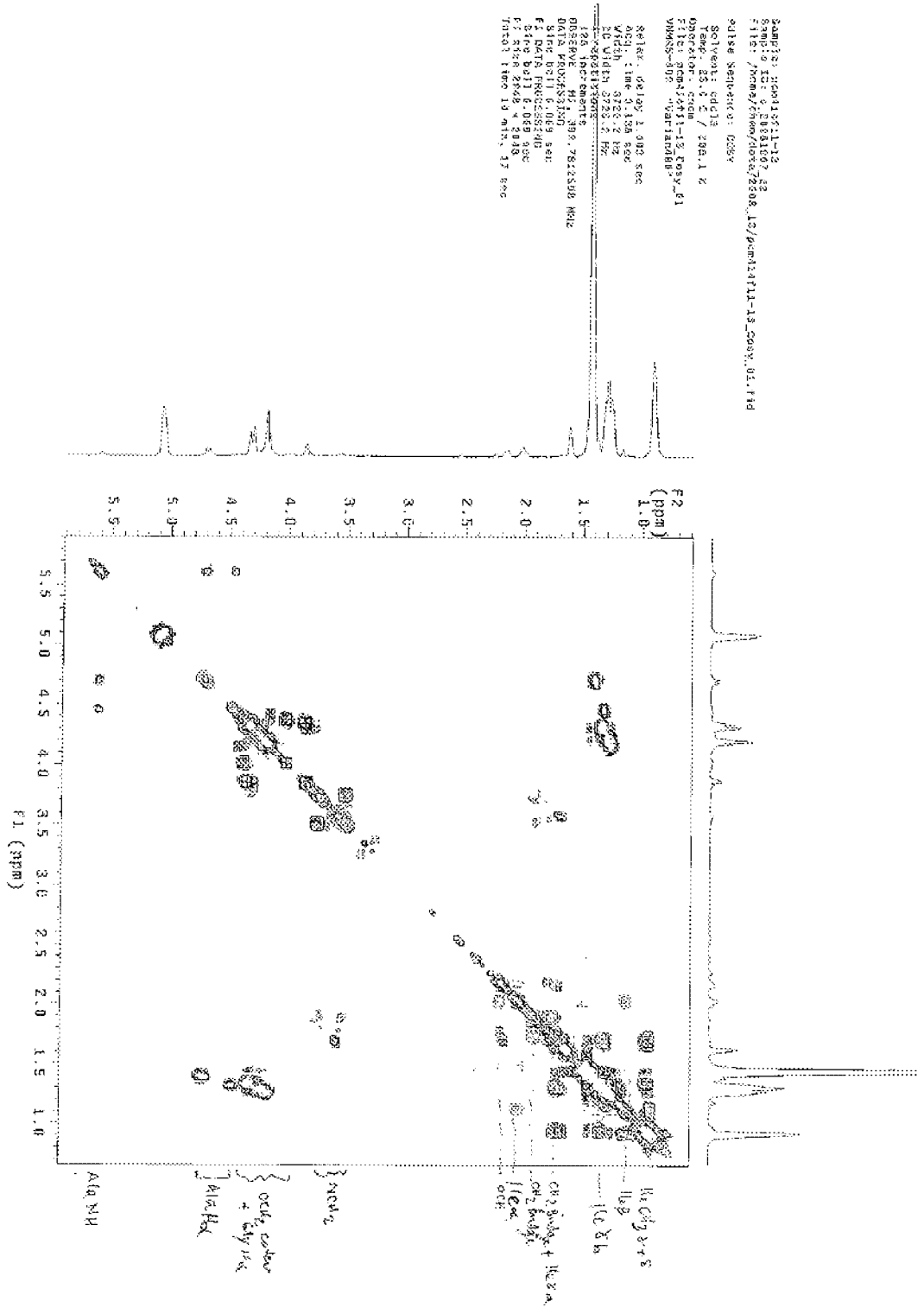

+Q1: 5.109 to 5.560 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 3.1e5 cps.
Intensity, cps
m/z, amu
478.1
534.2
434.2
273.3
556.2
383.2
572.1
456.2
496.1
324.2
573.2
709.2

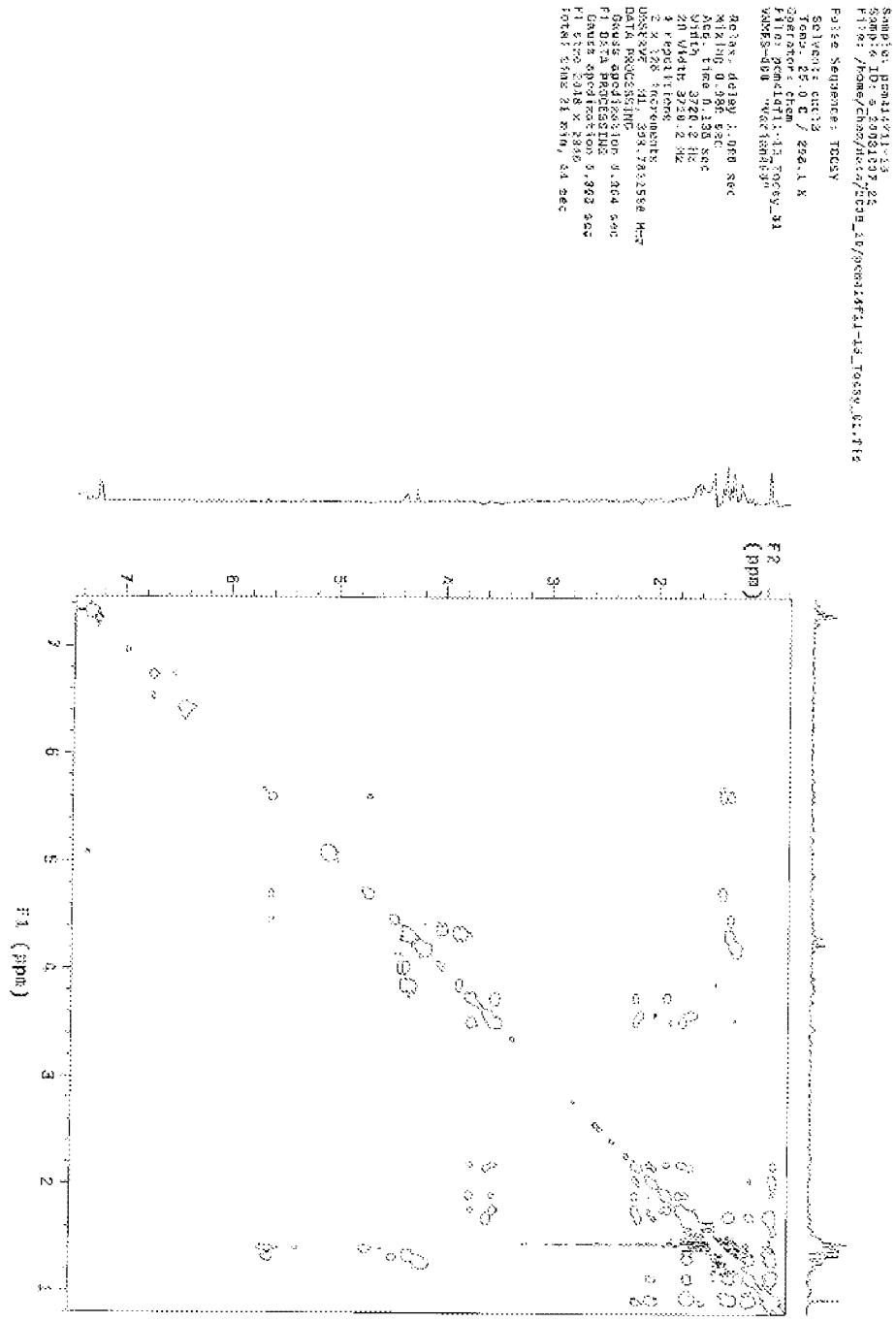

+Q1: 4.057 to 4.508 min from Sample 1 (pcm432f10fragtest) of pcm432f10fragtest.wiff (Turbo S...
Max. 5.8e5 cps.
Intensity, cps
m/z, amu
534.2
478.0
273.3
434.1
552.1
249.2
370.2
519.1
225.3
287.1
343.1
177.3
199.0
295.0
419.1
444.8
496.2
537.1
591.0
663.3
709.2
750.2
804.2
834.6
878.4
970.9

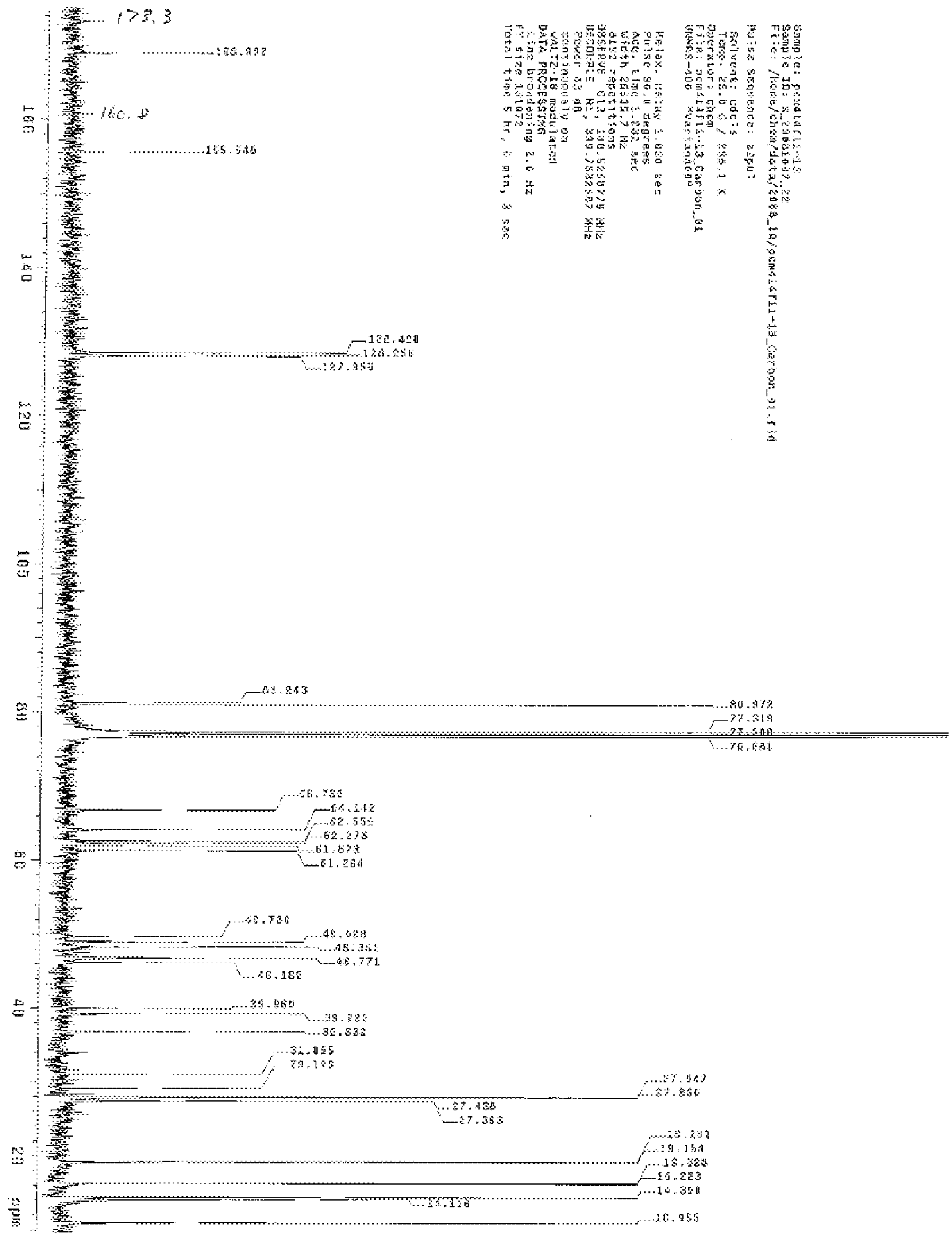
Appendix 9

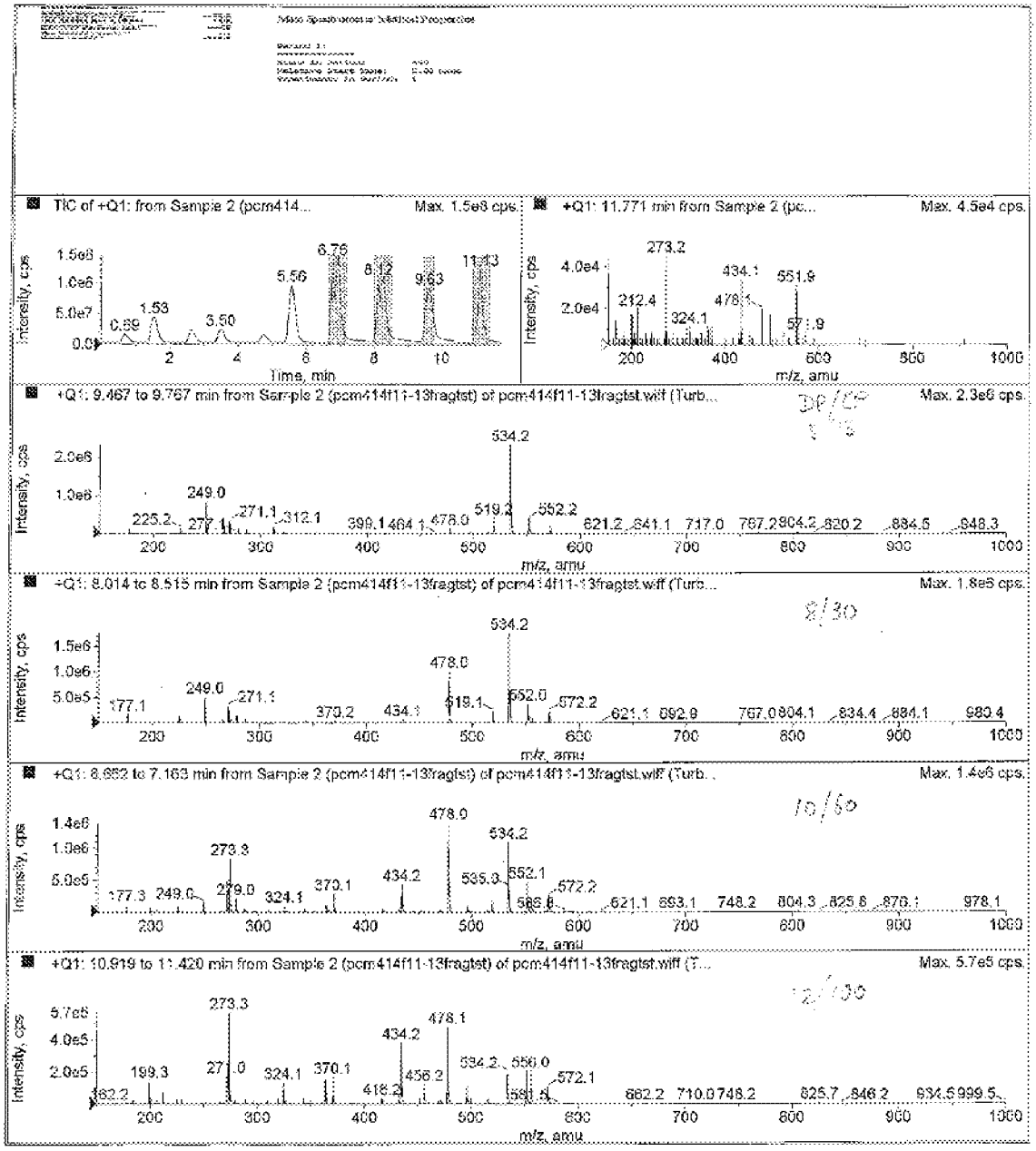
Data obtained for Compounds 3b

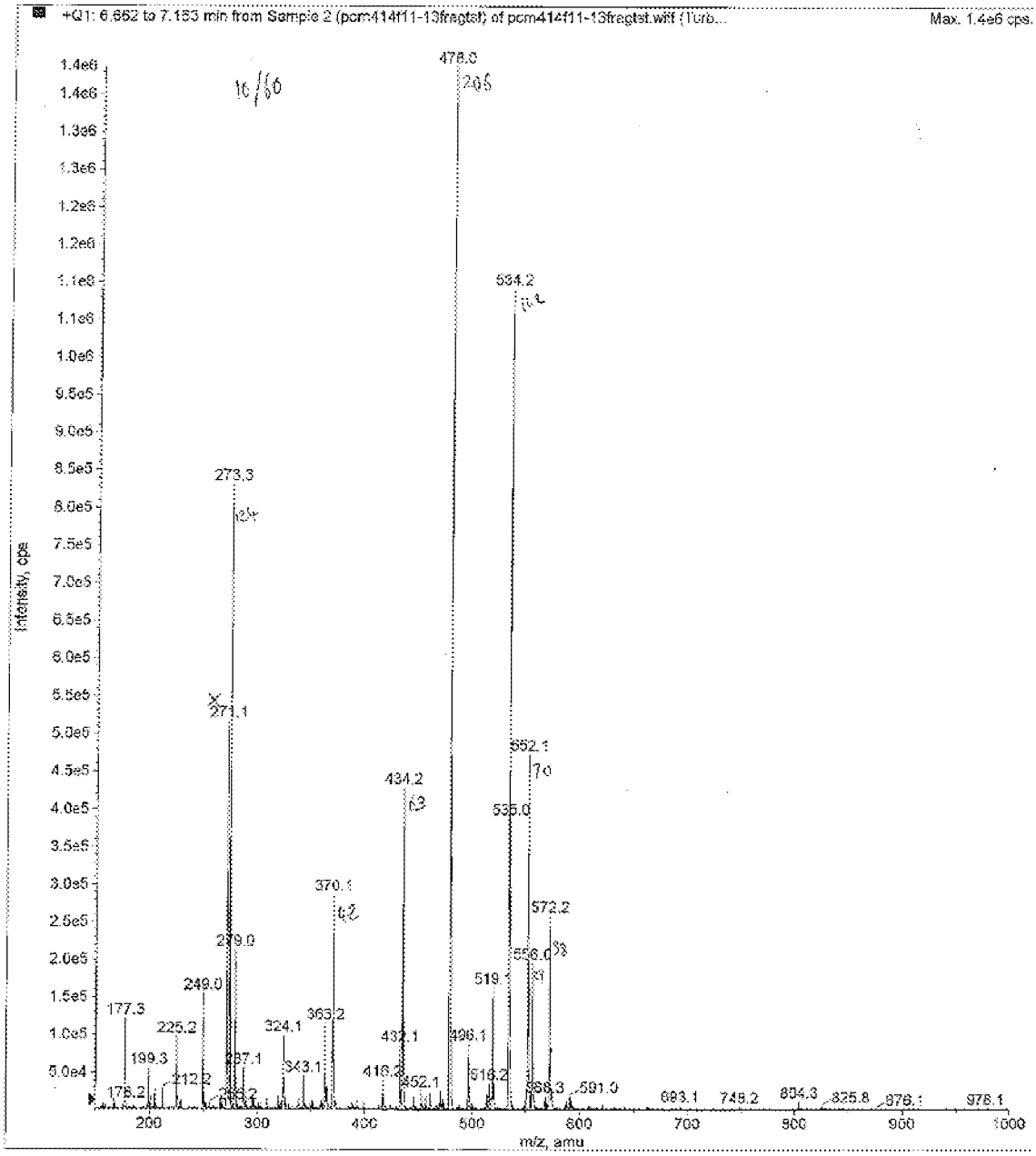

ppm
7
6
5
4
3
2

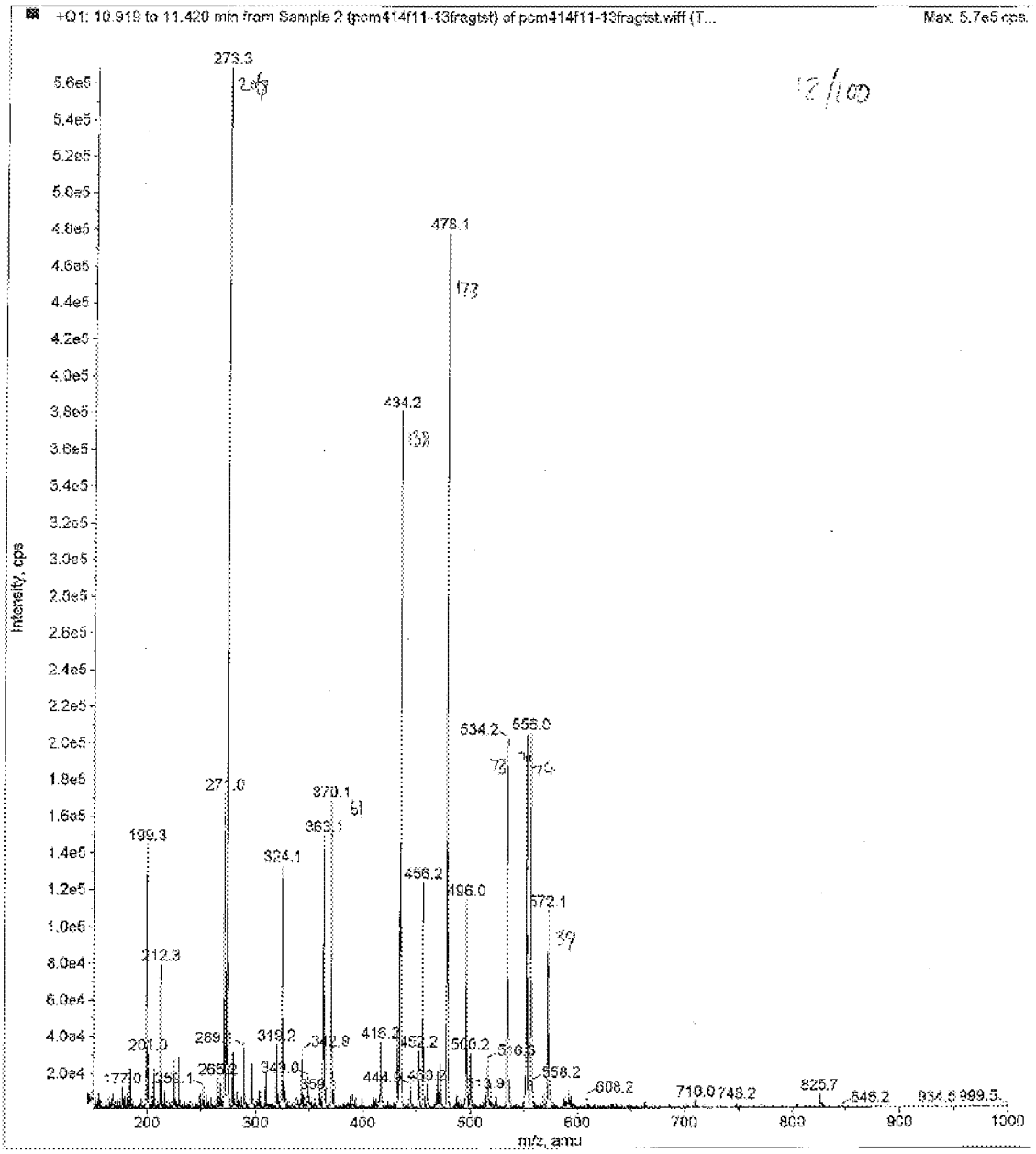



F2 (ppm)
F1 (ppm)



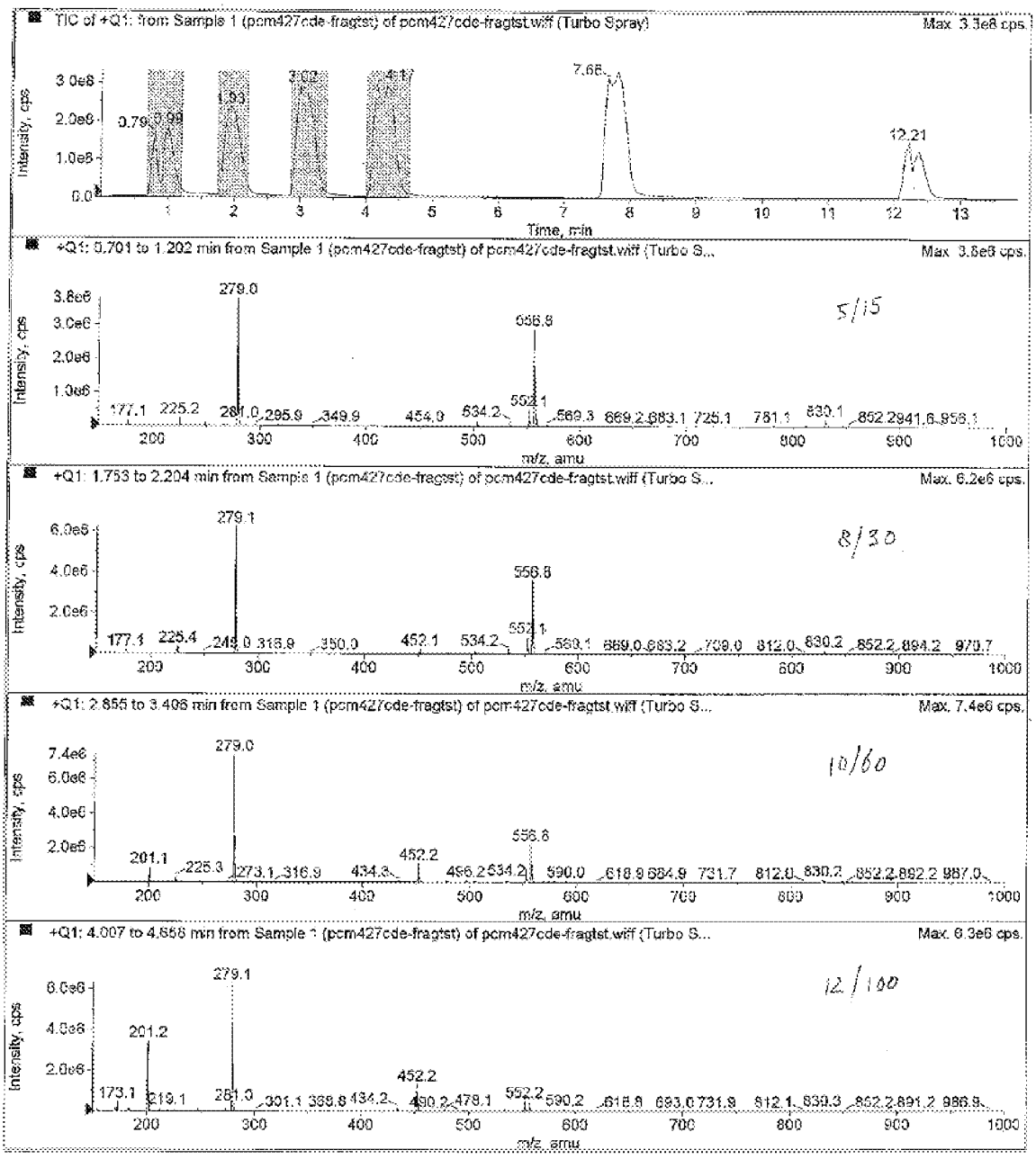

TIC of +Q1: from Sample 2 (pcm414...
Max. 1.5e8 cps.
Intensity, cps
Time, min
+Q1: 11.771 min from Sample 2 (pc...
Max. 4.5e4 cps.
m/z, amu
+Q1: 9.467 to 9.767 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (Turb...
Max. 2.3e6 cps.
+Q1: 8.014 to 8.515 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (Turb...
Max. 1.8e6 cps.
+Q1: 6.662 to 7.163 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (Turb...
Max. 1.4e6 cps.
+Q1: 10.919 to 11.420 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (T...
Max. 5.7e5 cps.

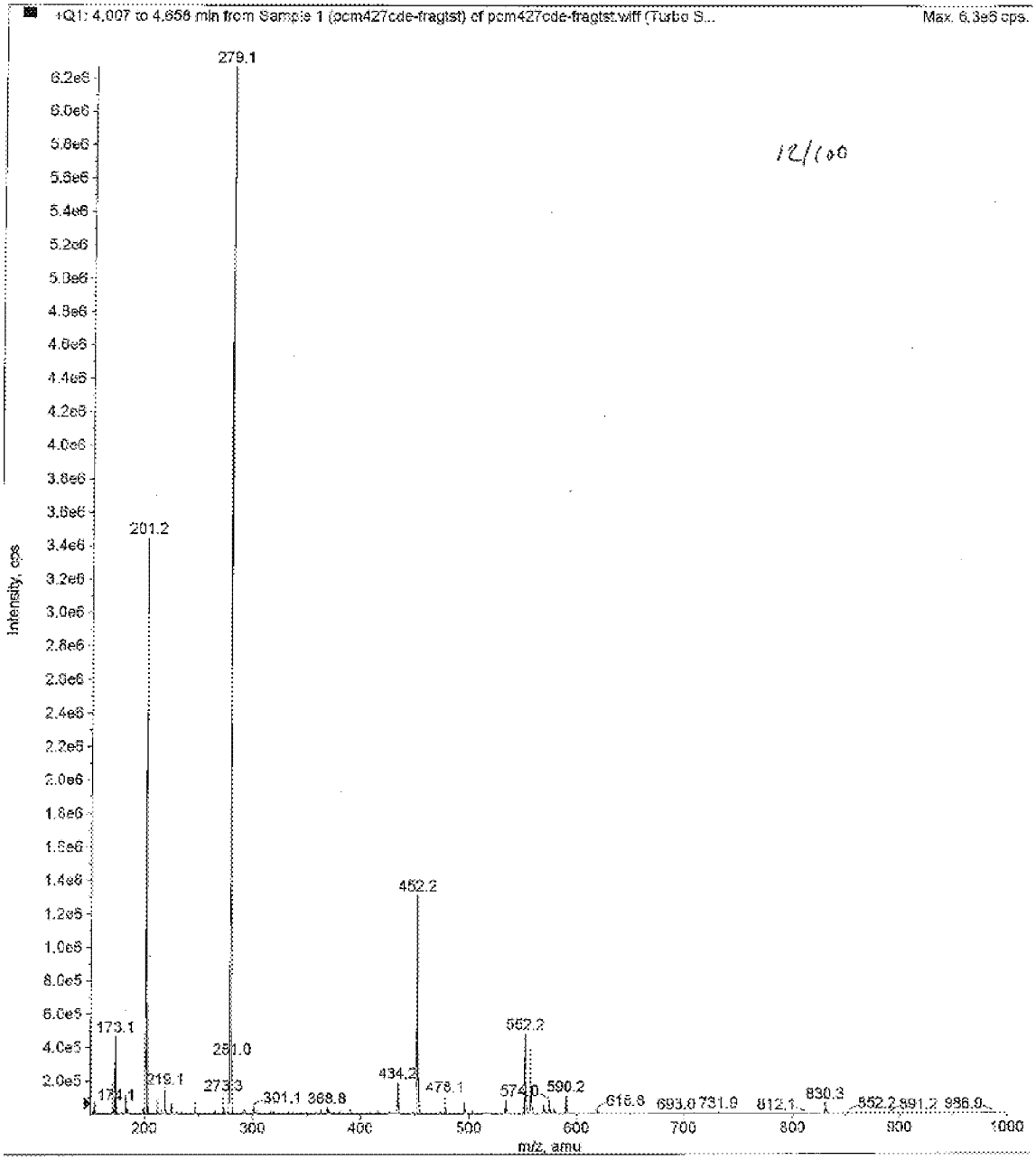

+Q1: 6.662 to 7.183 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (Turb...
Max. 1.4e6 cps.
Intensity, cps
m/z, amu
478.0
534.2
273.3
271.1
552.1
434.2
535.0
370.1
572.2
556.0
519
249.0
177.3
225.2
324.1
363.2
432.1
496.1
199.3
343.1
416.3
552.1
516.2
568.3
591.0
683.1
748.2
804.3
825.8
876.1
978.1

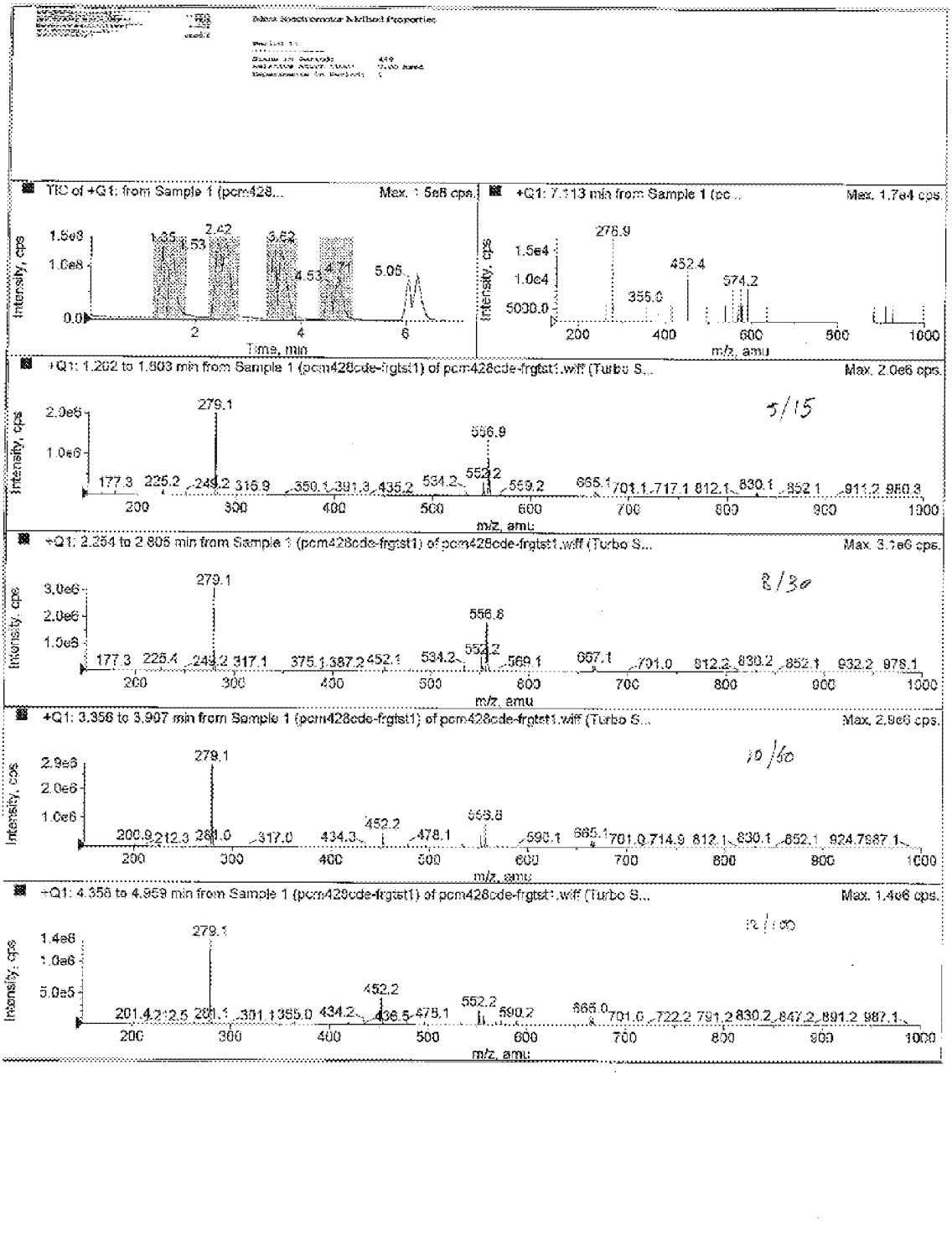

+Q1: 10.919 to 11.420 min from Sample 2 (pcm414f11-13fragtst) of pcm414f11-13fragtst.wiff (T...
Max. 5.7e5 cps.
Intensity, cps
m/z, amu
273.3
478.1
434.2
534.2
558.0
271.0
370.1
199.3
324.1
456.2
496.0
572.1
312.3
289.2
319.2
342.9
416.2
452.2
500.2
558.2
608.2
710.0
748.2
825.7
846.2
934.6
999.5

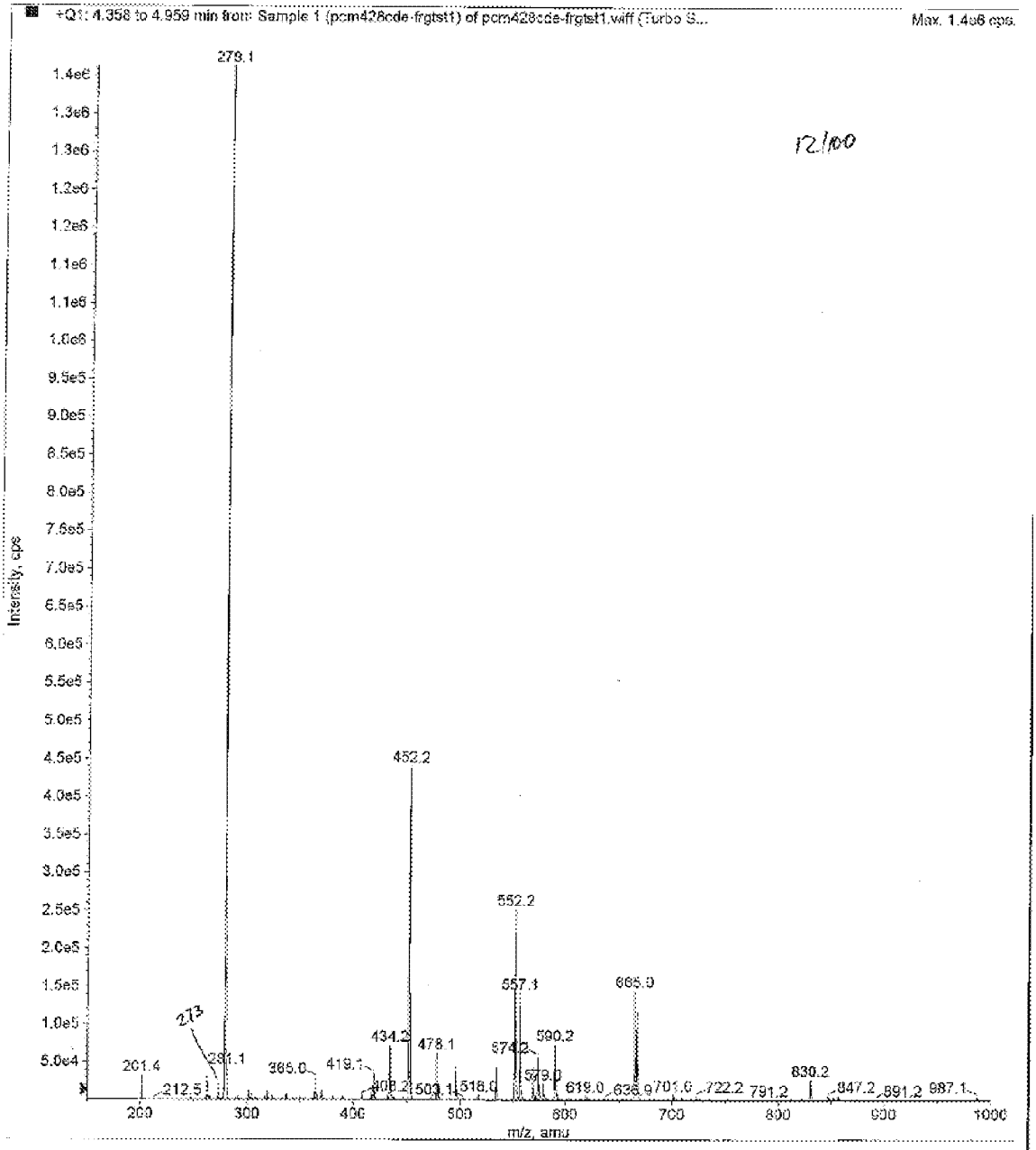
Appendix 10

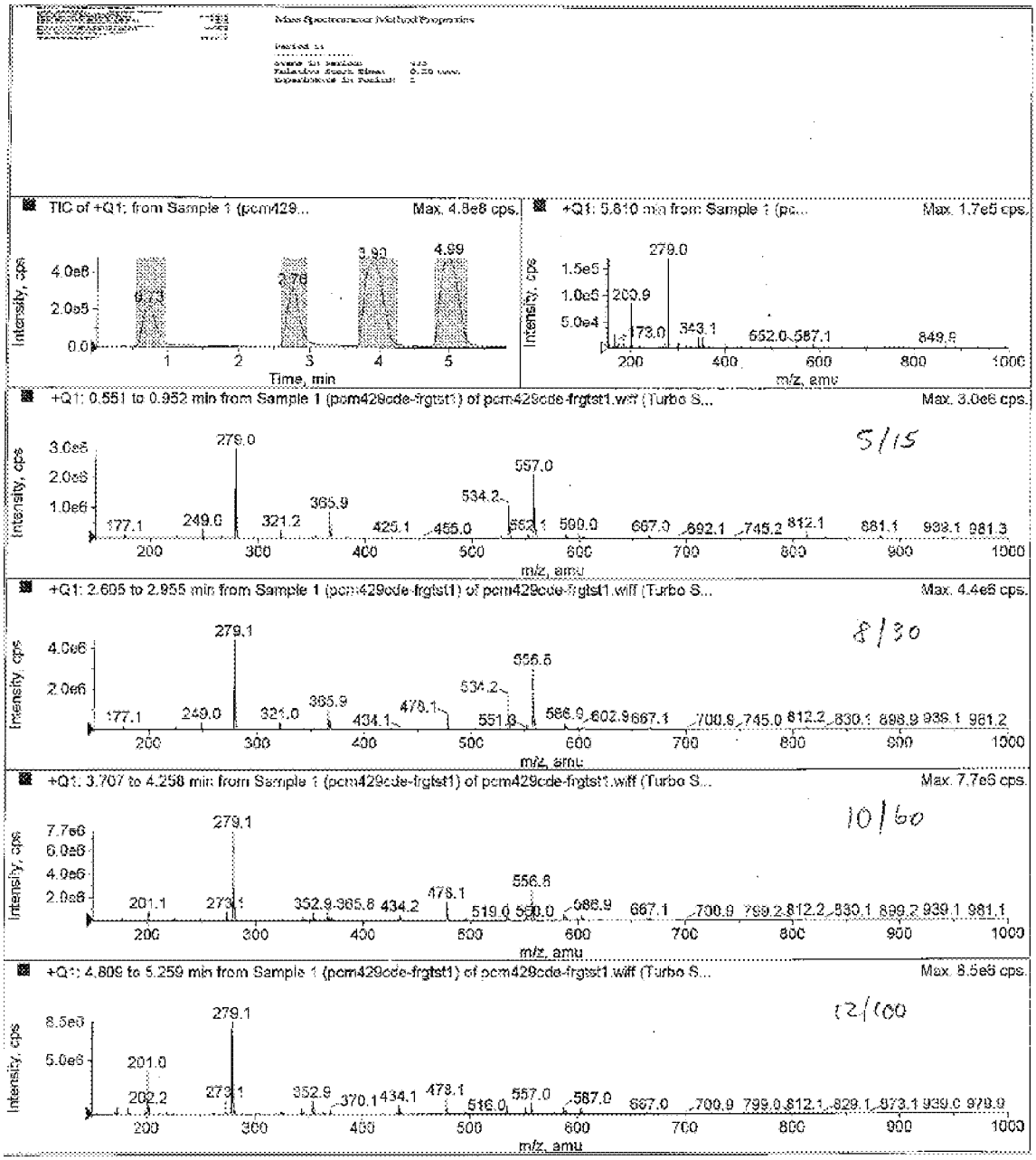
Factorial MS data from Factorial experiments

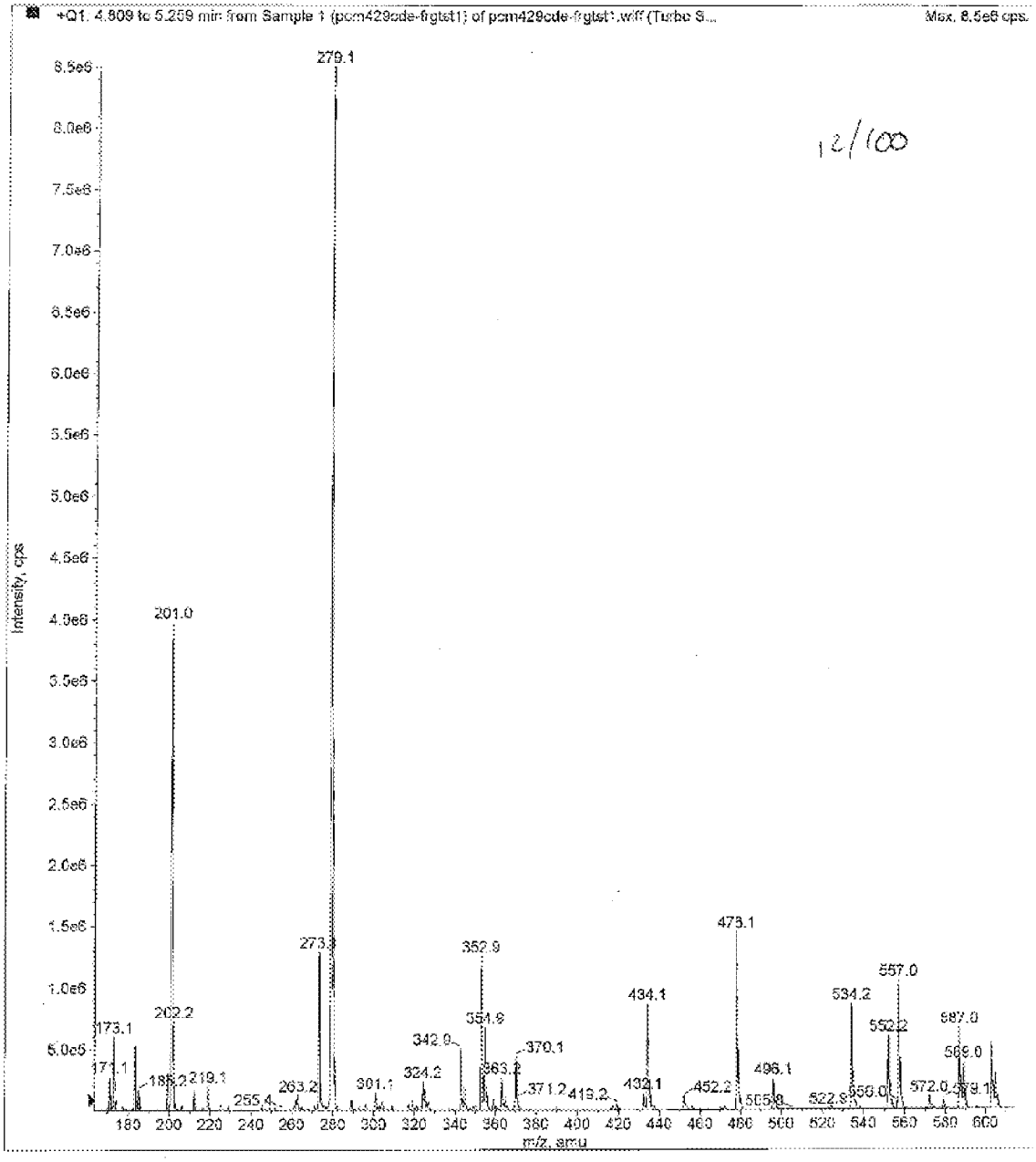

TIC of +Q1: from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo Spray)
Max. 3.3e8 cps.
Intensity, cps
Time, min
7.66
12.21
+Q1: 0.701 to 1.202 min from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo S...
Max. 3.8e8 cps.
279.0
556.8
5/15
m/z, amu
+Q1: 1.753 to 2.204 min from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo S...
Max. 6.2e6 cps.
279.1
556.8
8/30
+Q1: 2.855 to 3.406 min from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo S...
Max. 7.4e6 cps.
279.0
556.8
452.2
10/60
+Q1: 4.007 to 4.656 min from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo S...
Max. 6.3e6 cps.
279.1
201.2
452.2
12/100

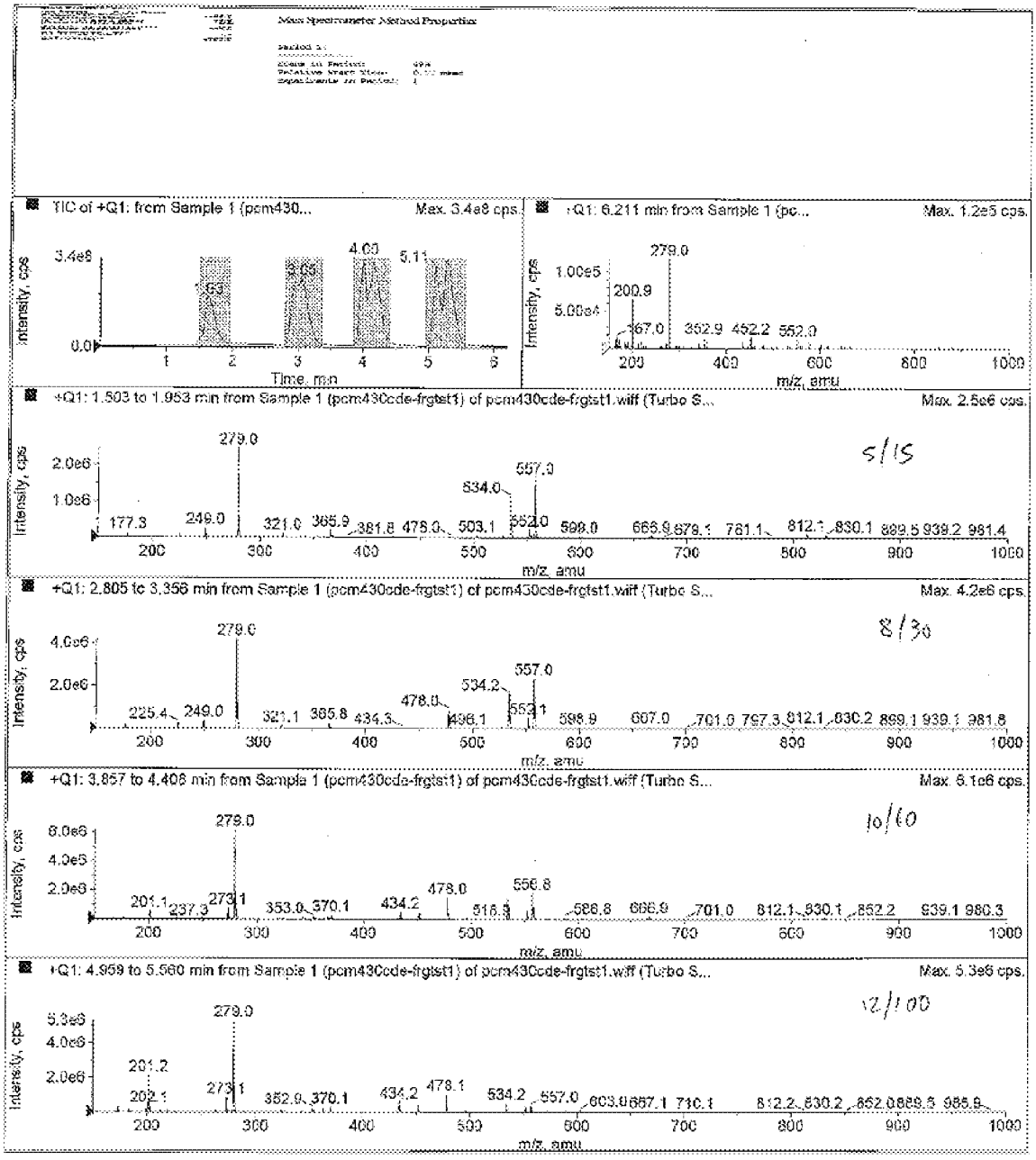

+Q1: 4.007 to 4.656 min from Sample 1 (pcm427cde-fragtst) of pcm427cde-fragtst.wiff (Turbo S...
Max. 6.3e6 cps.
12/100
Intensity, cps
m/z, amu
279.1
201.2
452.2
552.2
173.1
251.0
434.2
219.1
273.3
301.1
368.8
478.1
574.0
590.2
616.8
693.0
731.9
812.1
830.3
852.2
891.2
986.9

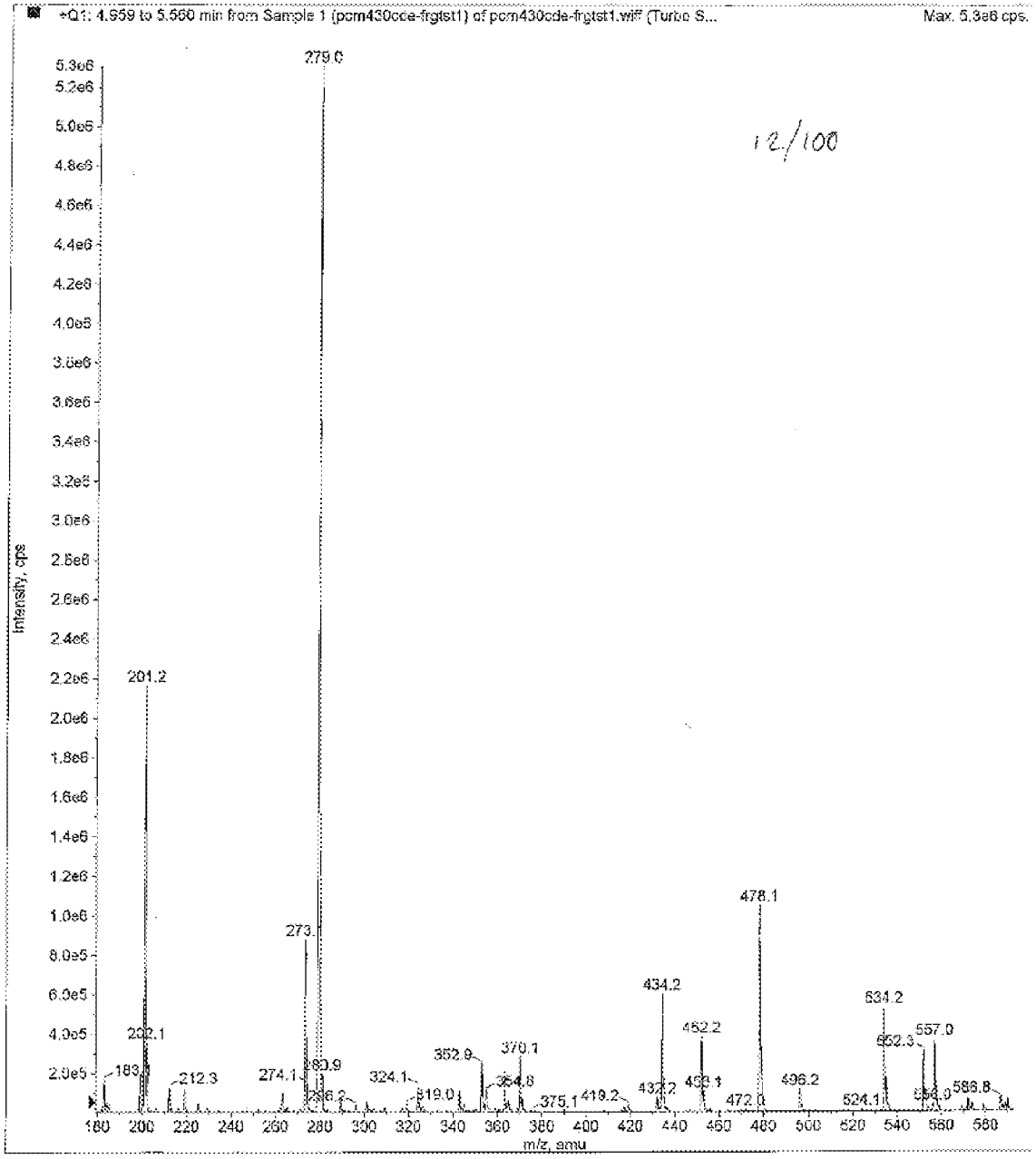

TIC of +Q1: from Sample 1 (pcm428...
Max. 1.5e8 cps.
Intensity, cps
Time, min
+Q1: 7.113 min from Sample 1 (pc...
Max. 1.7e4 cps.
276.9
452.4
574.2
355.0
m/z, amu
+Q1: 1.202 to 1.803 min from Sample 1 (pcm428cde-frgtst1) of pcm428cde-frgtst1.wiff (Turbo S...
Max. 2.0e6 cps.
279.1
556.9
5/15
+Q1: 2.354 to 2.805 min from Sample 1 (pcm428cde-frgtst1) of pcm428cde-frgtst1.wiff (Turbo S...
Max. 3.1e6 cps.
279.1
556.8
8/30
+Q1: 3.356 to 3.907 min from Sample 1 (pcm428cde-frgtst1) of pcm428cde-frgtst1.wiff (Turbo S...
Max. 2.9e6 cps.
279.1
452.2
10/60
+Q1: 4.358 to 4.959 min from Sample 1 (pcm428cde-frgtst1) of pcm428cde-frgtst1.wiff (Turbo S...
Max. 1.4e6 cps.
279.1
452.2
12/60

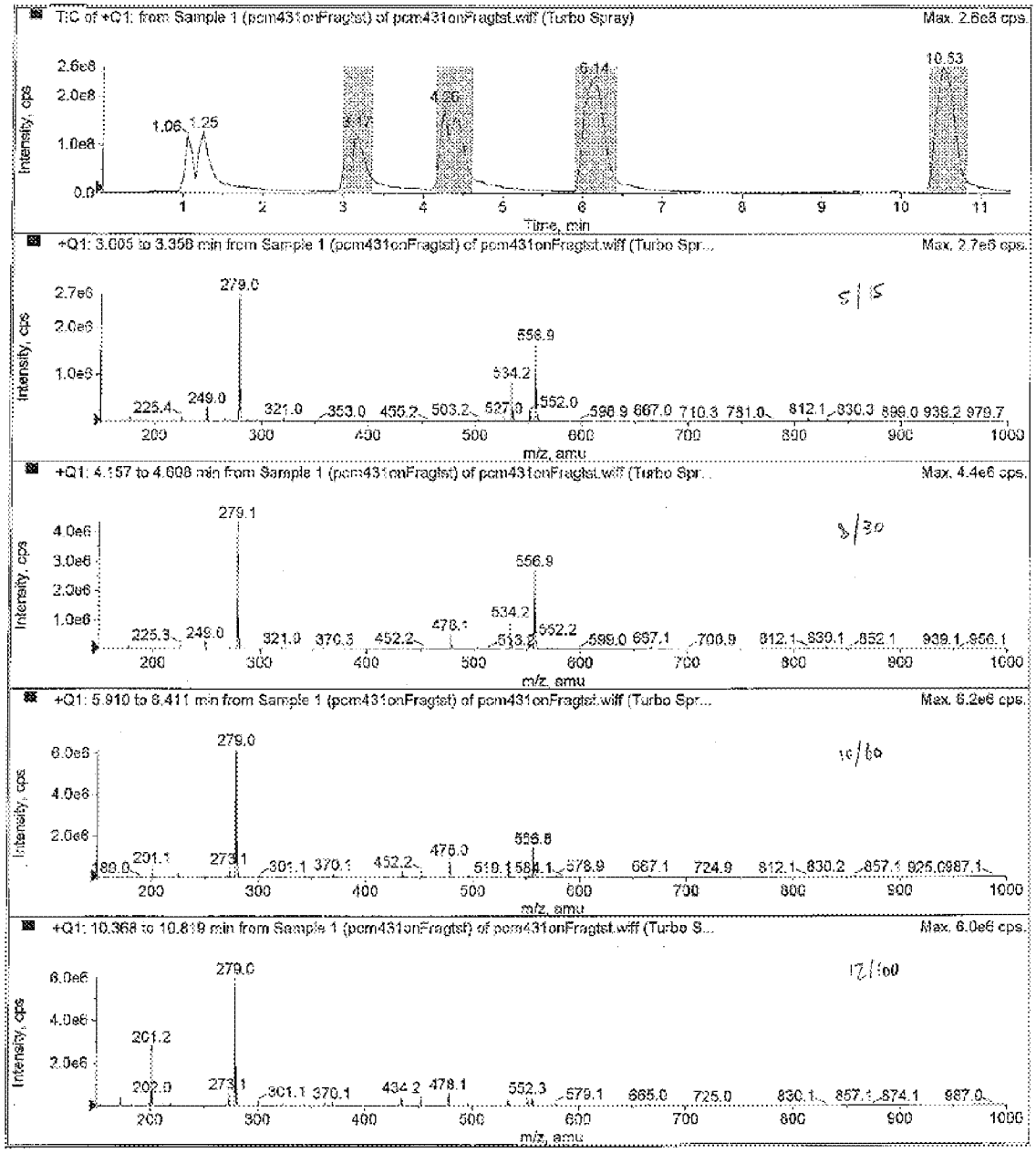

+Q1: 4.358 to 4.959 min from Sample 1 (pcm428cde-frgtst1) of pcm428cde-frgtst1.wiff (Turbo S...
Max. 1.4e6 cps.
278.1
452.2
552.2
557.1
665.9
590.2
434.2
478.1
574.2
830.2
273
201.4
212.5
281.1
365.0
419.1
408.2
503.1
518.0
579.0
619.0
636.9
701.0
722.2
791.2
847.2
891.2
987.1
Intensity, cps
m/z, amu
12/100

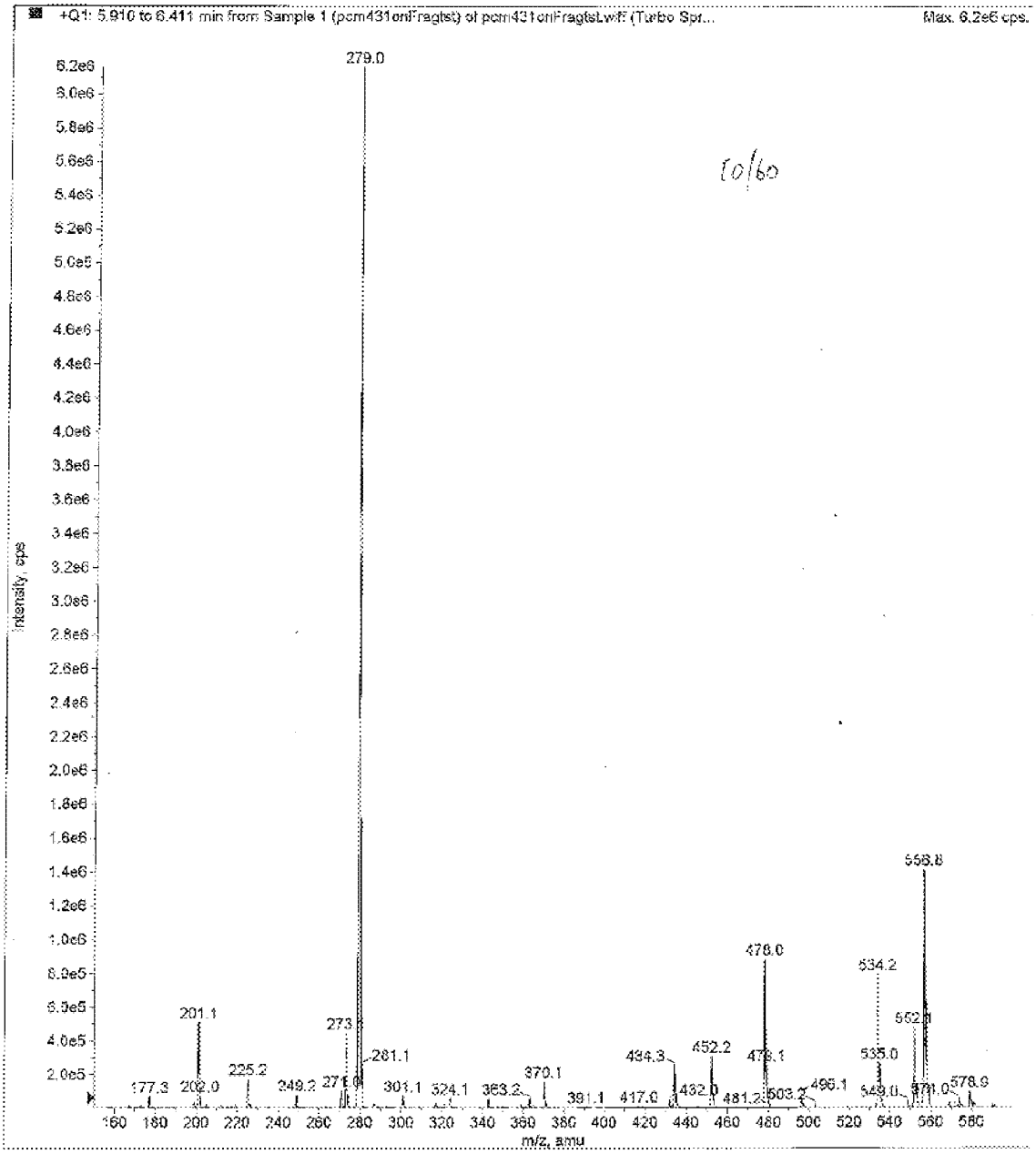

TIC of +Q1: from Sample 1 (pcm429...
Max. 4.8e8 cps.
Intensity, cps
Time, min
+Q1: 5.610 min from Sample 1 (pc...
Max. 1.7e5 cps.
279.0
+Q1: 0.551 to 0.952 min from Sample 1 (pcm429cde-frgtst1) of pcm429cde-frgtst1.wiff (Turbo S...
Max. 3.0e6 cps.
5/15
+Q1: 2.605 to 2.955 min from Sample 1 (pcm429cde-frgtst1) of pcm429cde-frgtst1.wiff (Turbo S...
Max. 4.4e6 cps.
8/30
+Q1: 3.707 to 4.258 min from Sample 1 (pcm429cde-frgtst1) of pcm429cde-frgtst1.wiff (Turbo S...
Max. 7.7e6 cps.
10/60
+Q1: 4.809 to 5.259 min from Sample 1 (pcm429cde-frgtst1) of pcm429cde-frgtst1.wiff (Turbo S...
Max. 8.5e6 cps.
12/100
m/z, amu

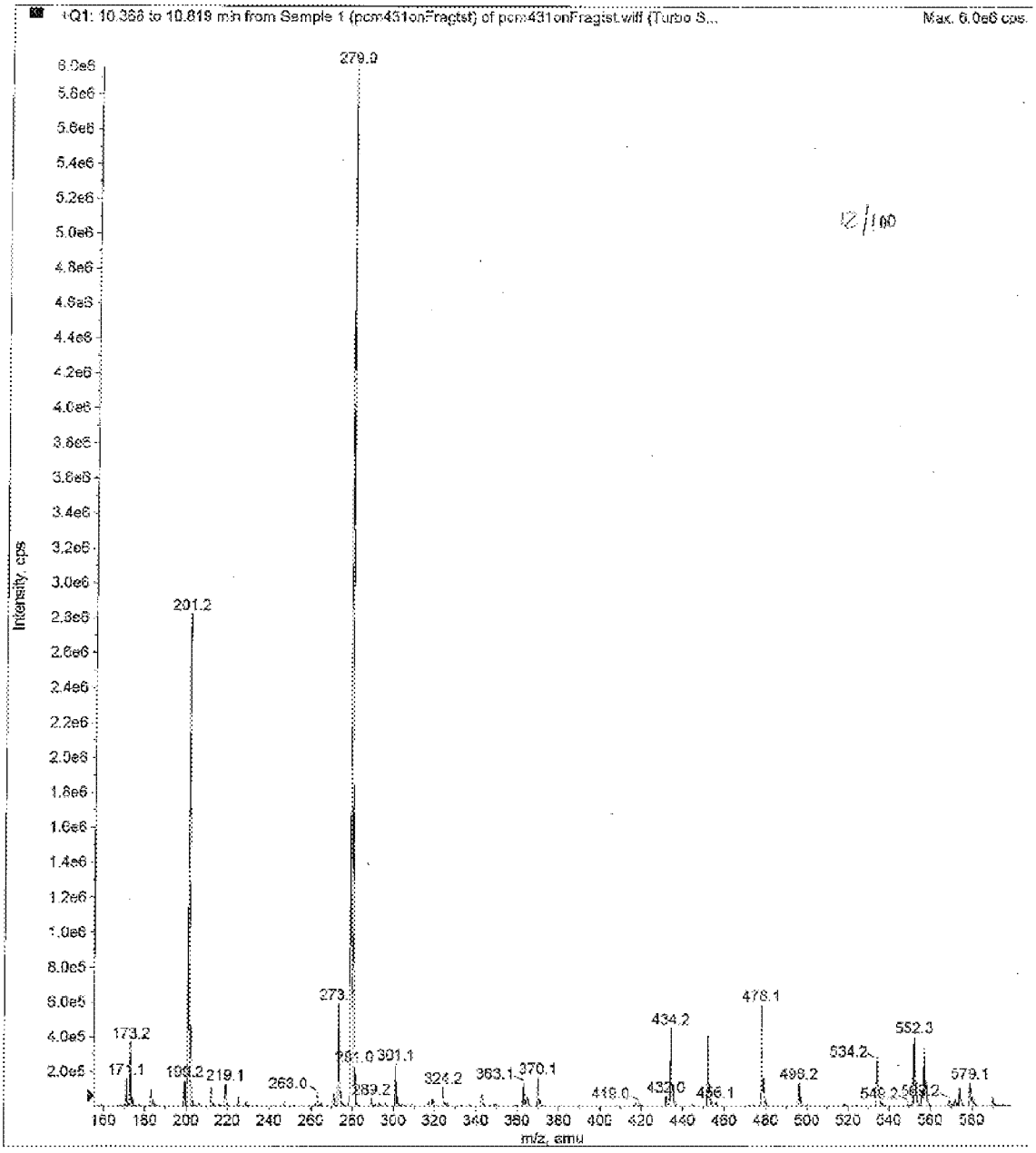

+Q1, 4.809 to 5.259 min from Sample 1 (pcm429cde-frgtst1) of pcm429cde-frgtst1.wiff (Turbo S...
Max. 8.5e6 cps.
12/100
Intensity, cps
m/z, amu
279.1
201.0
202.2
273.3
352.9
354.9
434.1
473.1
557.0
534.2
552.2
587.0
569.0
342.9
370.1
496.1
173.1
324.2
301.1
263.2
219.1
432.1
452.2
572.0

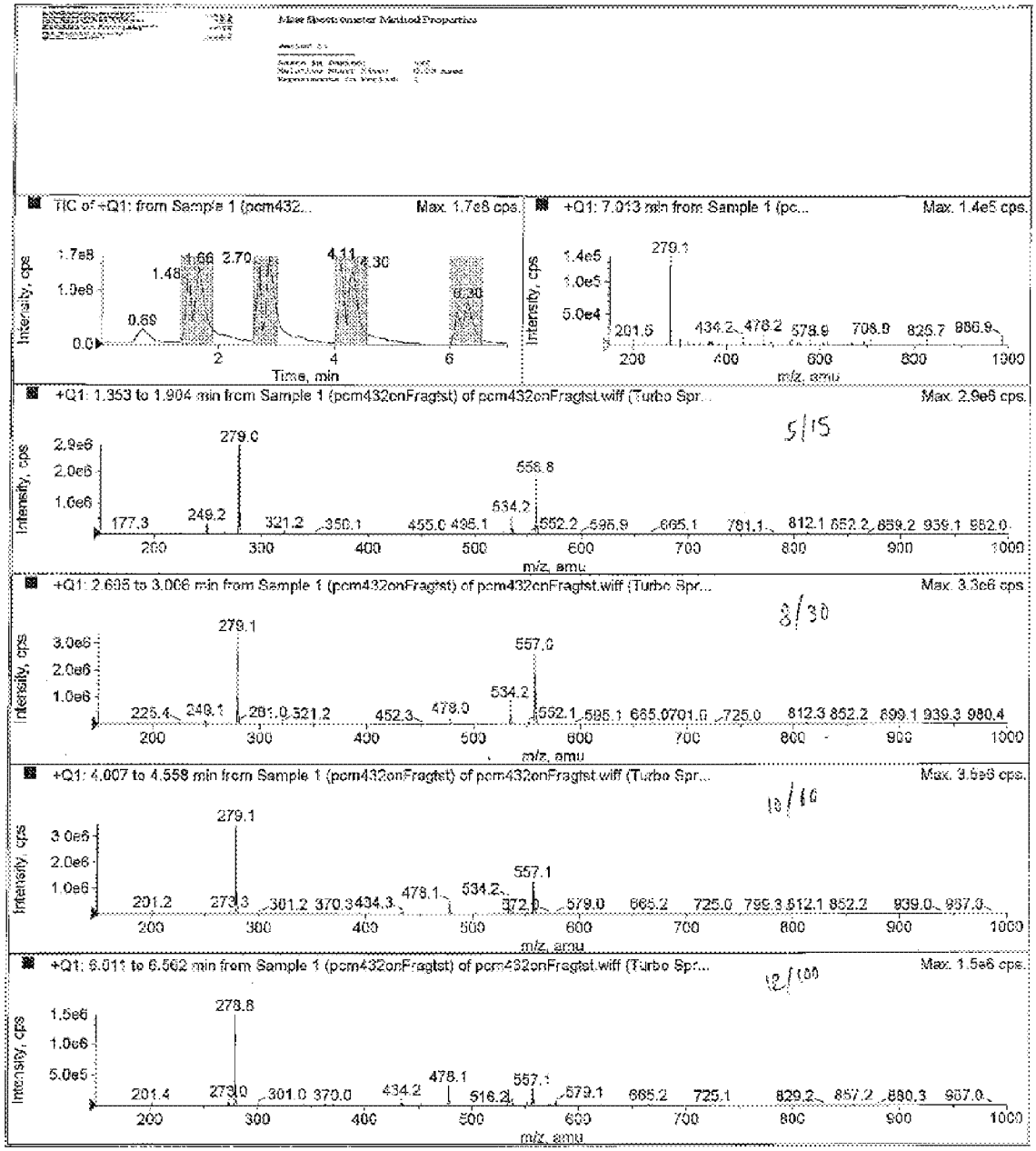

TIC of +Q1: from Sample 1 (pcm430...
Max. 3.4e8 cps.
Intensity, cps
Time, min
+Q1: 6.211 min from Sample 1 (pc...
Max. 1.2e5 cps.
m/z, amu
+Q1: 1.503 to 1.983 min from Sample 1 (pcm430cde-frgtst1) of pcm430cde-frgtst1.wiff (Turbo S...
Max. 2.5e6 cps.
5/15
+Q1: 2.805 to 3.356 min from Sample 1 (pcm430cde-frgtst1) of pcm430cde-frgtst1.wiff (Turbo S...
Max. 4.2e6 cps.
8/30
+Q1: 3.857 to 4.408 min from Sample 1 (pcm430cde-frgtst1) of pcm430cde-frgtst1.wiff (Turbo S...
Max. 8.1e6 cps.
10/10
+Q1: 4.959 to 5.560 min from Sample 1 (pcm430cde-frgtst1) of pcm430cde-frgtst1.wiff (Turbo S...
Max. 5.3e6 cps.
12/100

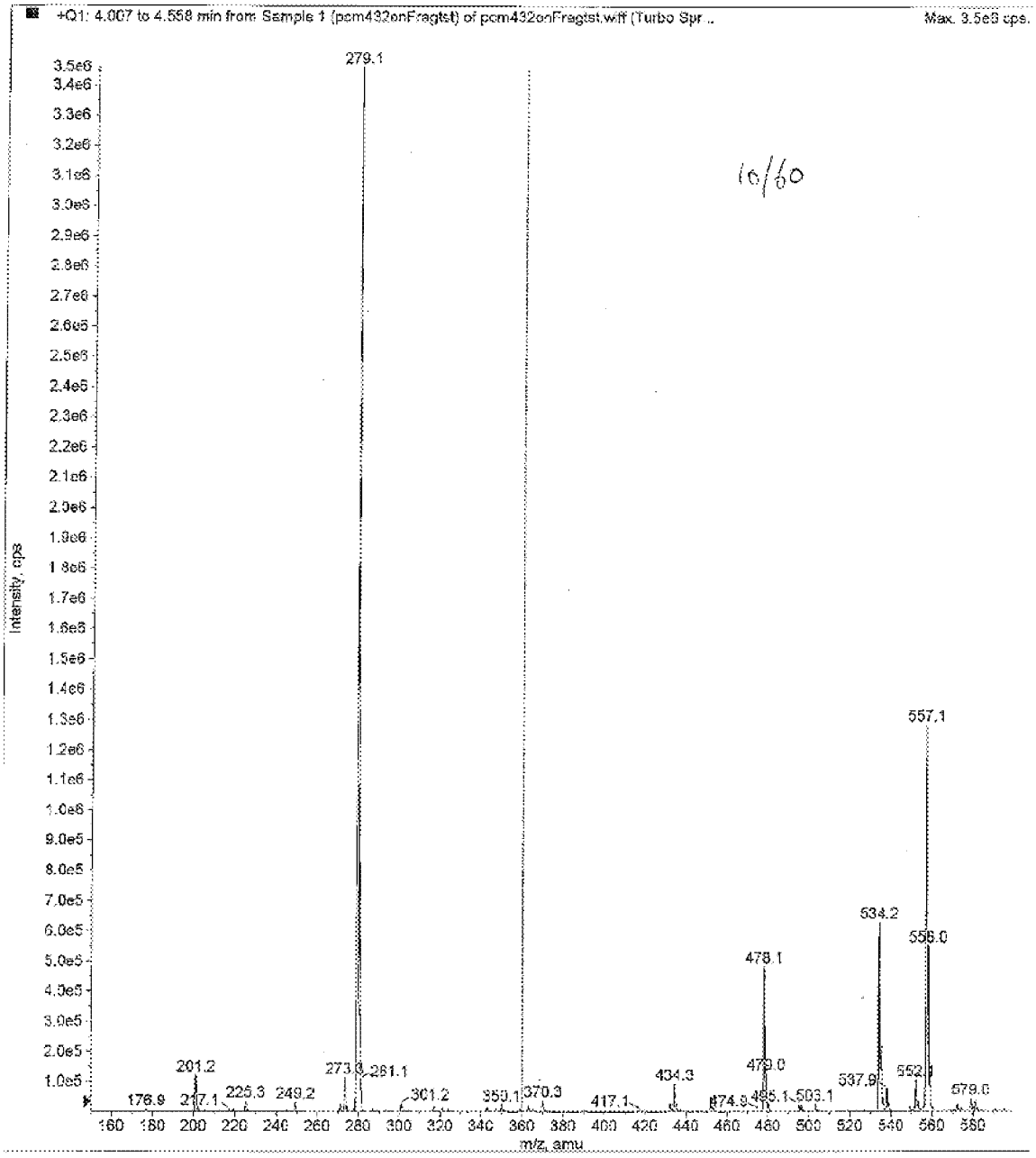

+Q1: 4.959 to 5.560 min from Sample 1 (pcm430cde-frgtst1) of pcm430cde-frgtst1.wiff (Turbo S...
Max. 5.3e6 cps.
12/100
Intensity, cps
m/z, amu
279.0
201.2
273.
478.1
434.2
534.2
462.2
557.0
352.9
370.1
202.1
183.
212.3
274.1
324.1
496.2
586.8
5.3e6
5.2e6
5.0e6
4.8e6
4.6e6
4.4e6
4.2e6
4.0e6
3.8e6
3.6e6
3.4e6
3.2e6
3.0e6
2.8e6
2.6e6
2.4e6
2.2e6
2.0e6
1.8e6
1.6e6
1.4e6
1.2e6
1.0e6
8.0e5
6.0e5
4.0e5
2.0e5
180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580

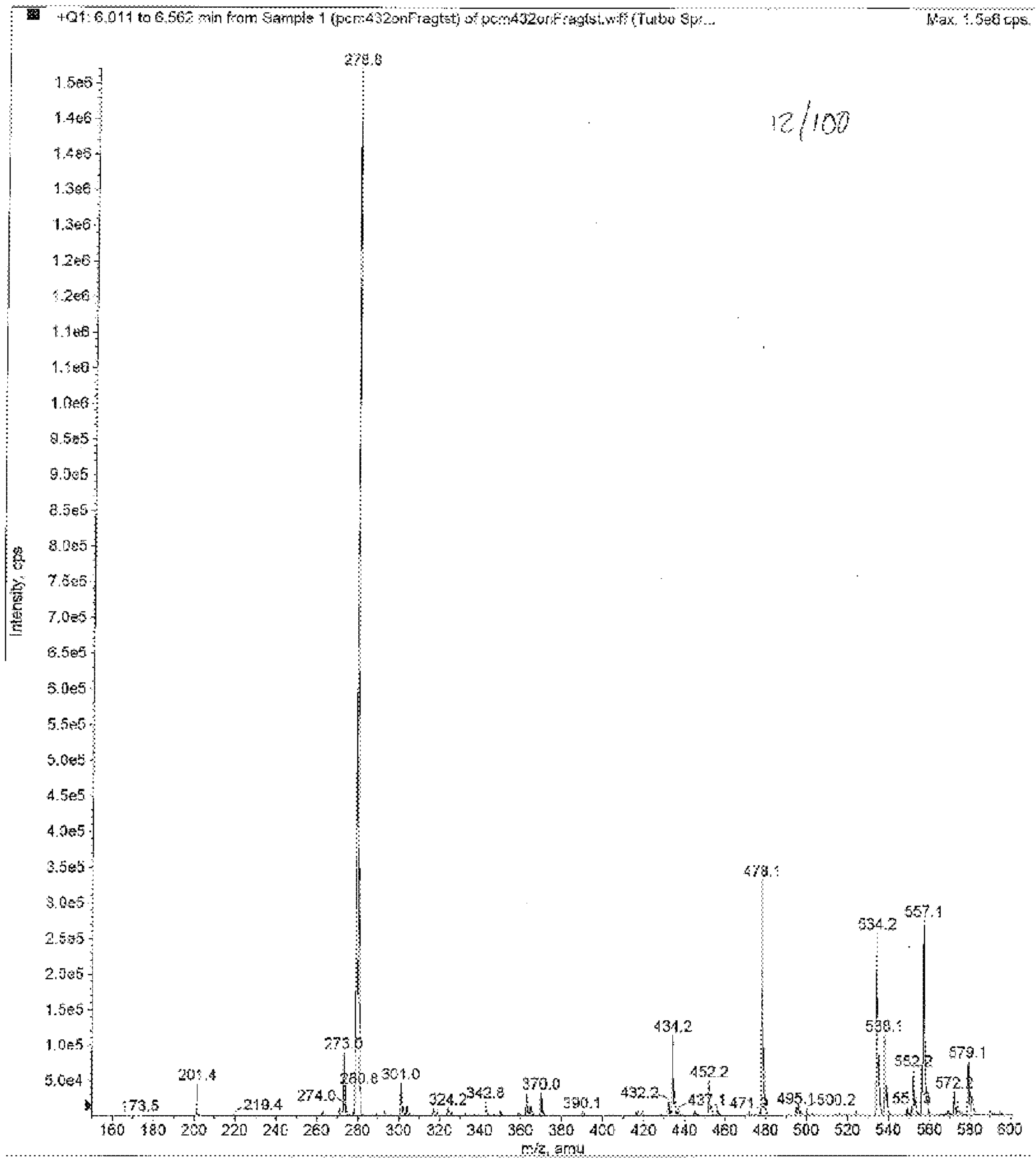

TIC of +Q1: from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo Spray)
Max. 2.6e8 cps.
Intensity, cps
Time, min
1.06
1.25
4.26
6.14
10.53
+Q1: 3.605 to 3.358 min from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo Spr...
Max. 2.7e6 cps.
279.0
556.9
534.2
552.0
m/z, amu
+Q1: 4.157 to 4.608 min from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo Spr...
Max. 4.4e6 cps.
279.1
556.9
534.2
552.2
m/z, amu
+Q1: 5.910 to 6.411 min from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo Spr...
Max. 6.2e6 cps.
279.0
556.8
m/z, amu
+Q1: 10.368 to 10.819 min from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo S...
Max. 6.0e6 cps.
279.0
201.2
m/z, amu


+Q1: 5.910 to 6.411 min from Sample 1 (pcm431onFragts) of pcm431onFragtsLwiff (Turbo Spr...
Max. 6.2e6 cps.
Intensity, cps
m/z, amu
279.0
201.1
273
281.1
225.2
370.1
434.3
452.2
478.0
534.2
558.8
10/60


Q1: 10.366 to 10.819 min from Sample 1 (pcm431onFragtst) of pcm431onFragtst.wiff (Turbo S...
Max. 6.0e6 cps.
12/100
Intensity, cps
m/z, amu
279.0
201.2
273
173.2
434.2
478.1
552.3
534.2
498.2
579.1
301.1
324.2
363.1
370.1
219.1
269.0
289.2
416.0
486.1


TIC of +Q1: from Sample 1 (pcm432...
Max. 1.7e8 cps.
Intensity, cps
Time, min
+Q1: 7.013 min from Sample 1 (pc...
Max. 1.4e5 cps.
279.1
m/z, amu
+Q1: 1.353 to 1.904 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr...
Max. 2.9e6 cps.
5/15
279.0
556.8
534.2
m/z, amu
+Q1: 2.686 to 3.006 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr...
Max. 3.3e6 cps.
3/30
279.1
557.0
534.2
m/z, amu
+Q1: 4.007 to 4.558 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr...
Max. 3.6e6 cps.
18/60
279.1
557.1
534.2
m/z, amu
+Q1: 6.011 to 6.562 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr...
Max. 1.5e6 cps.
12/100
278.8
478.1
557.1
534.2
m/z, amu

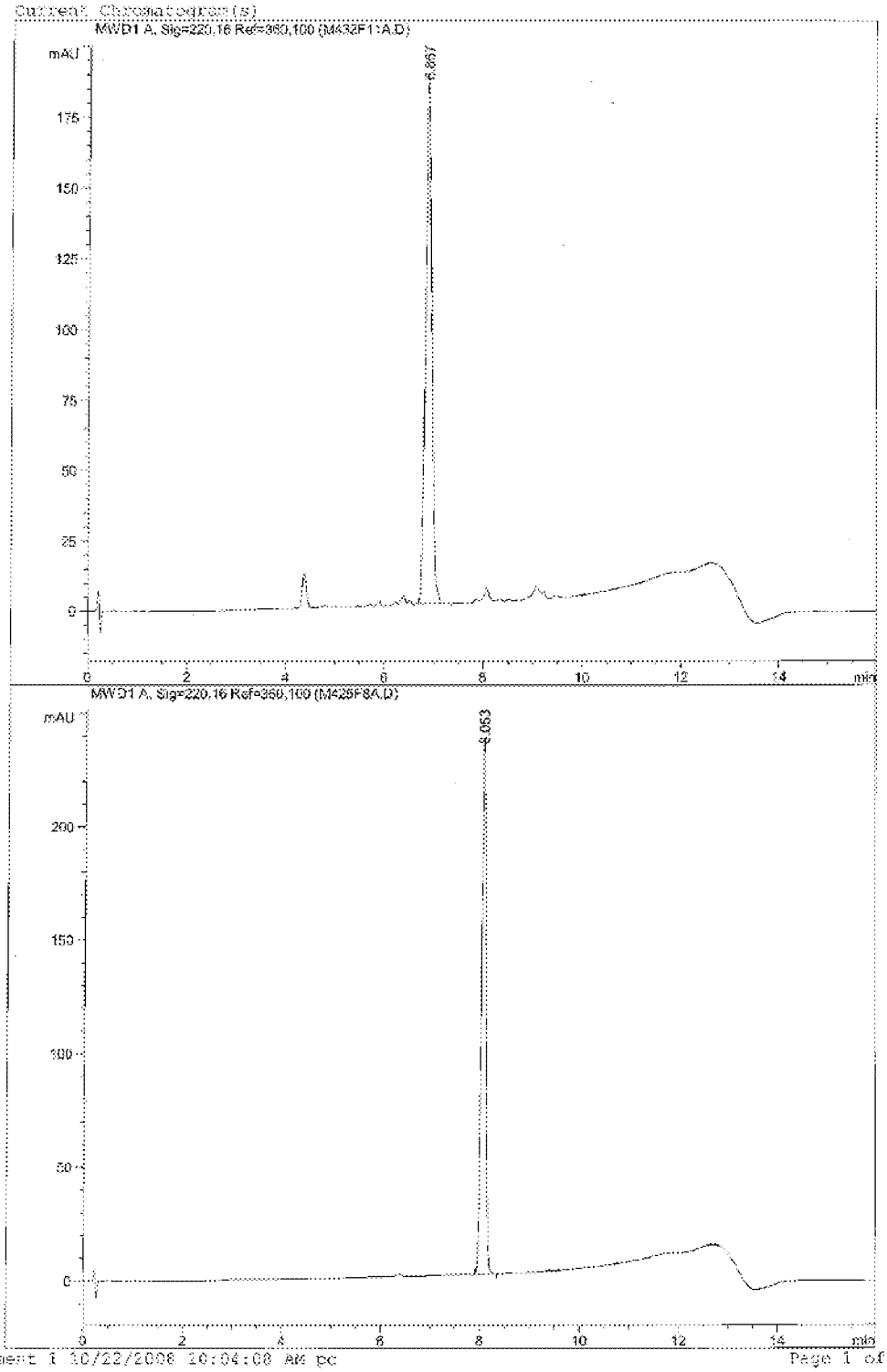

+Q1: 4.007 to 4.558 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr ..
Max. 3.5e6 cps.
Intensity, cps
m/z, amu
279.1
557.1
534.2
556.0
478.1
201.2
273.0
281.1
434.3
537.9
552.1
579.0
301.2
370.3
417.1
10/60


+Q1: 6.011 to 6.562 min from Sample 1 (pcm432onFragtst) of pcm432onFragtst.wiff (Turbo Spr...
Max. 1.5e6 cps.
12/100
Intensity, cps
m/z, amu
278.8
478.1
534.2
557.1
434.2
273.0
201.4
301.0
370.0
452.2
579.1

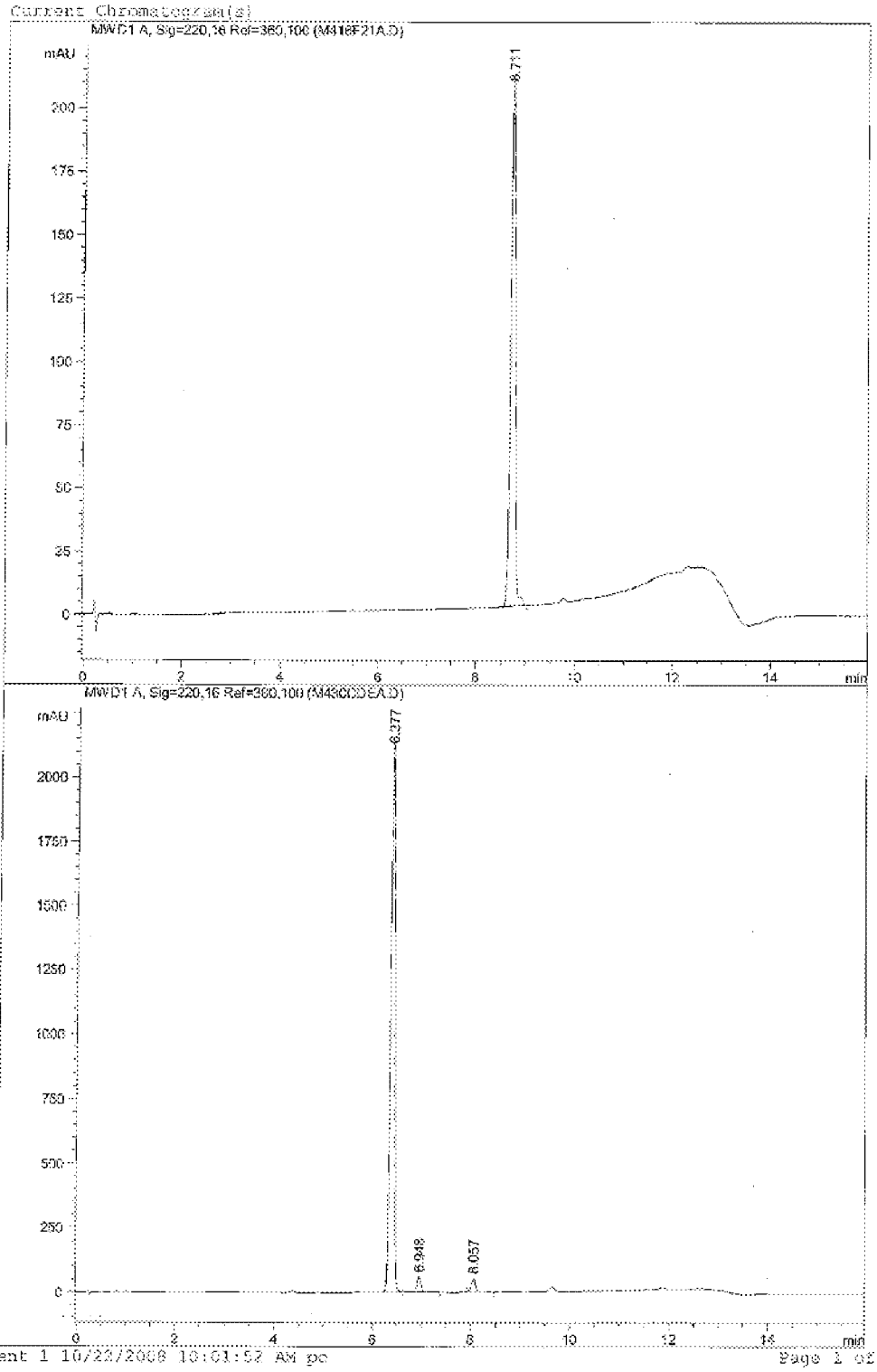
APPENDIX 11

HPLC Pure Compound and Co-injection Traces

Print of window 38: Current Chromatogram(s)



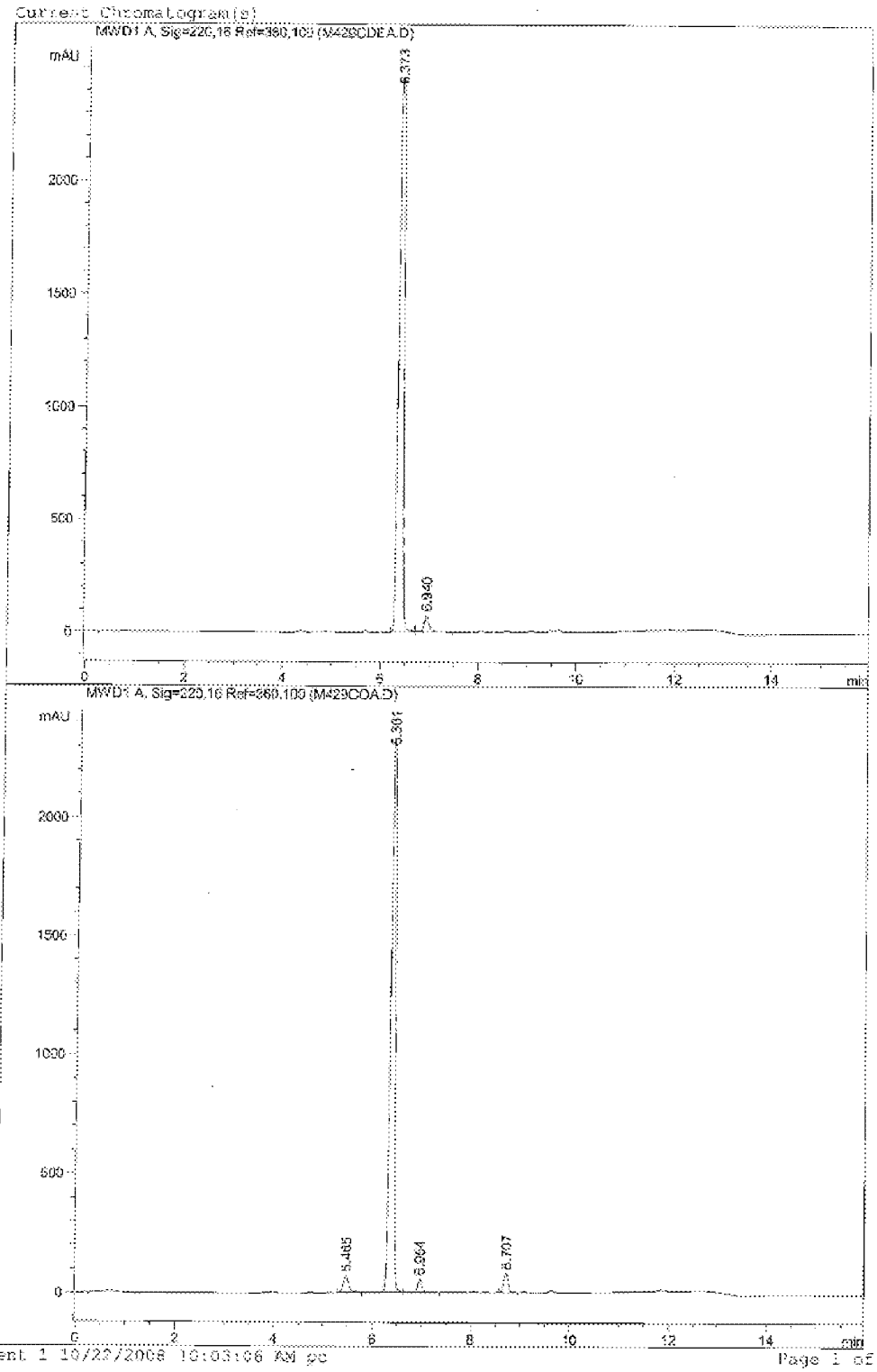
Print of Window 38: Current Chromatogram(s)



Instrument 1 10/22/2008 10:01:57 AM pc

Page 1 of 1

Print of window 38: Current Chromatogram(s)

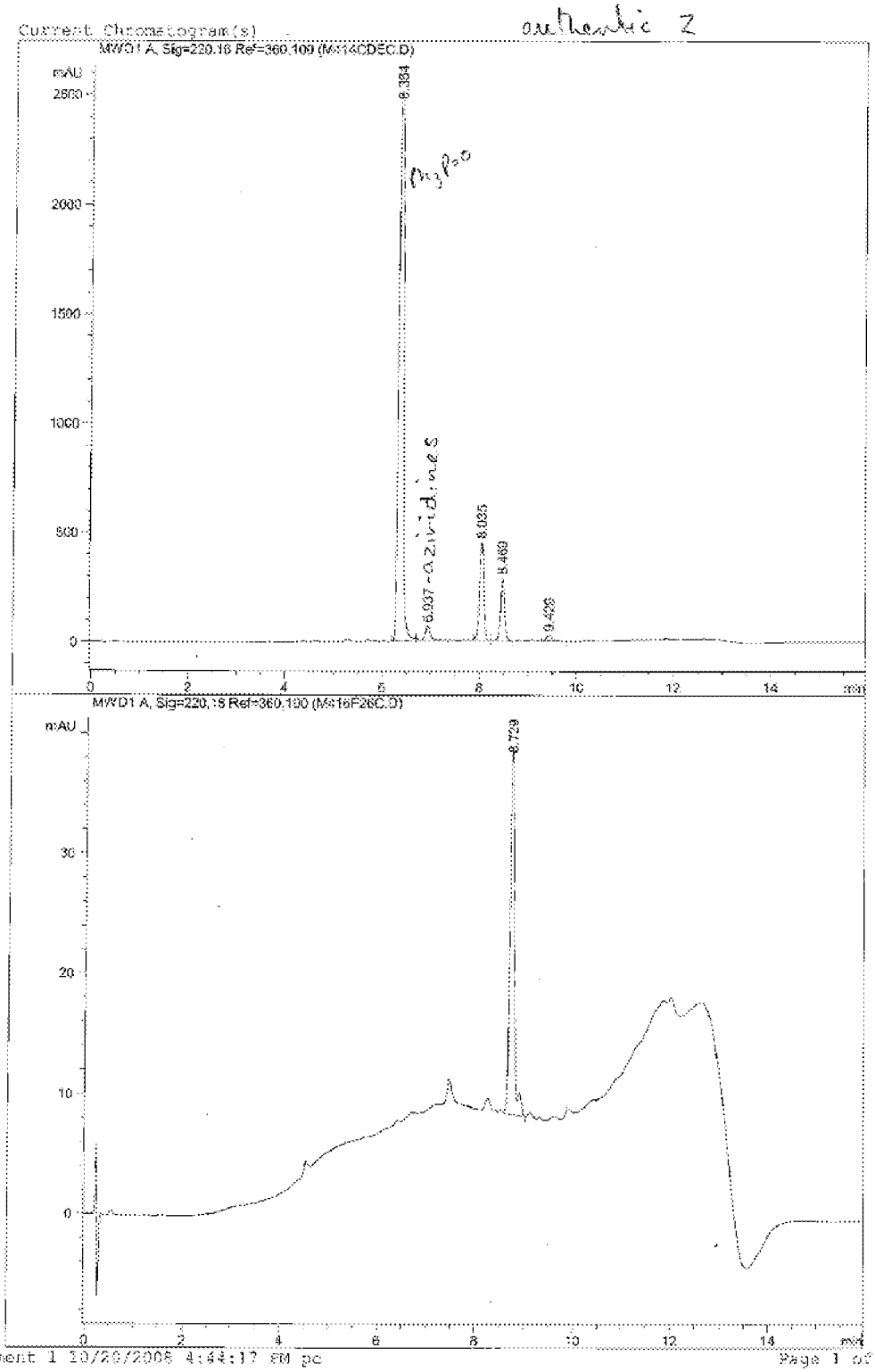


APPENDIX 12

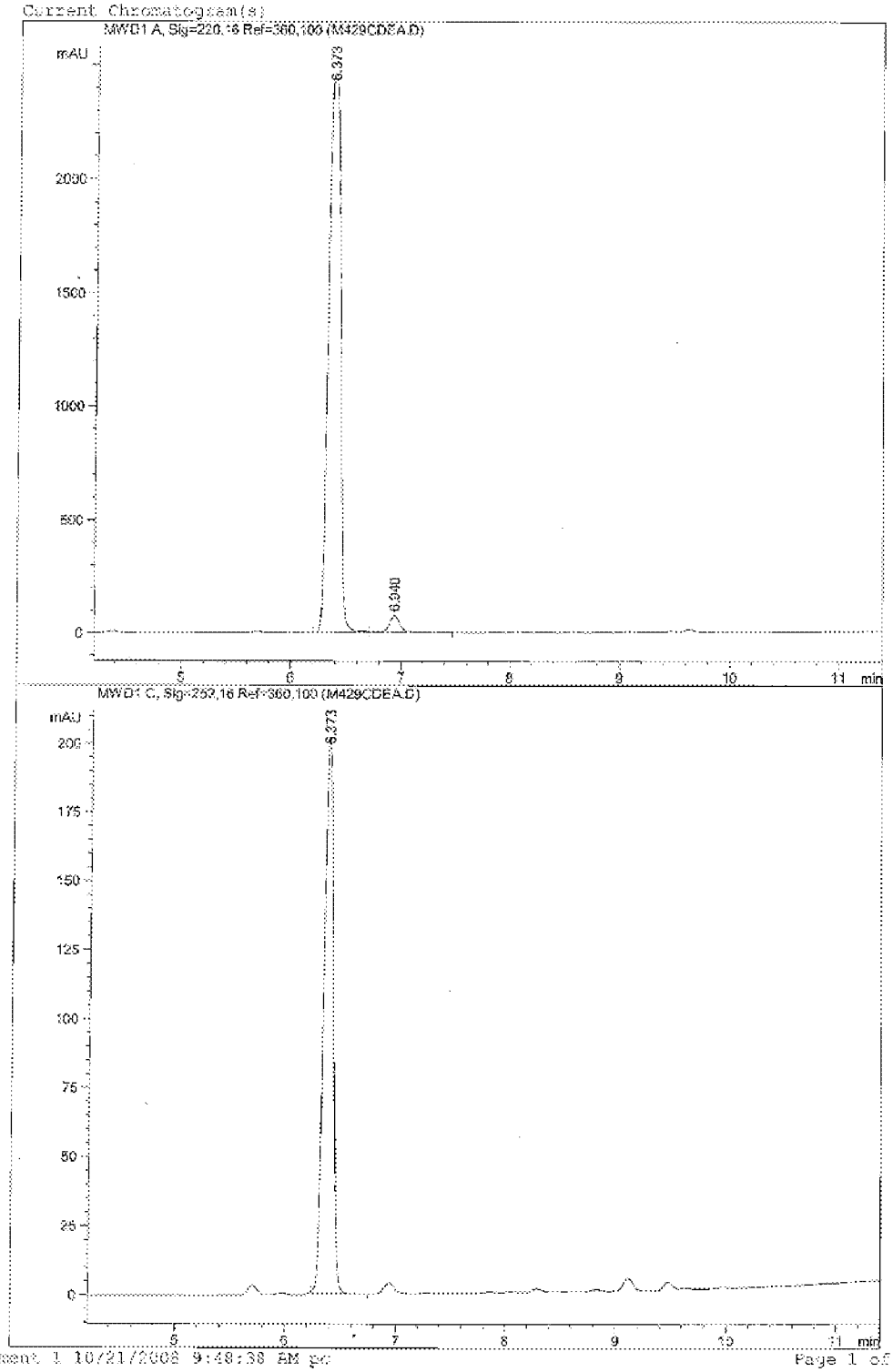
HPLC Reaction Mixture traces

Print of Window 38: Current Chromatogram(s)

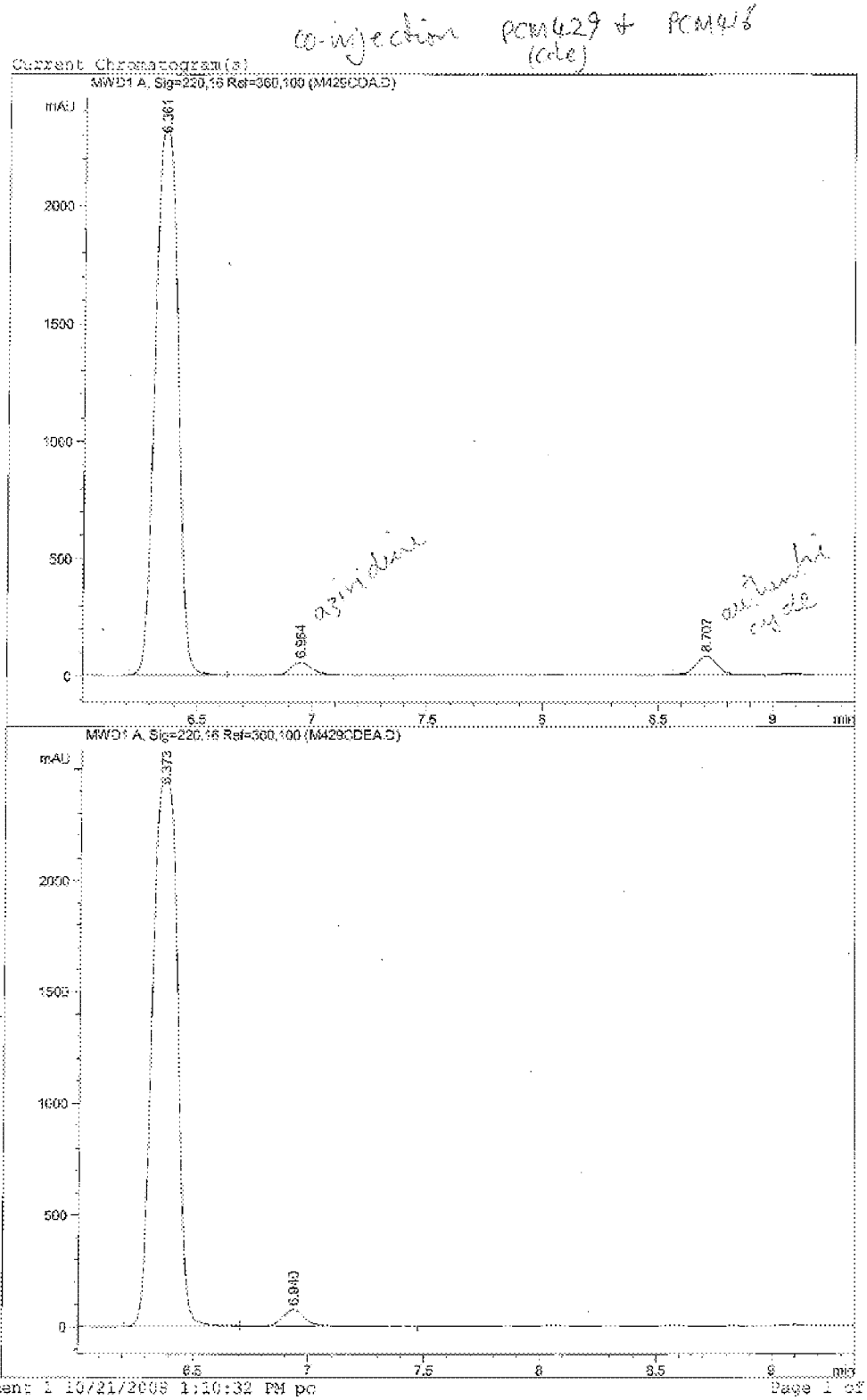
peptide cde compared to authentic 2



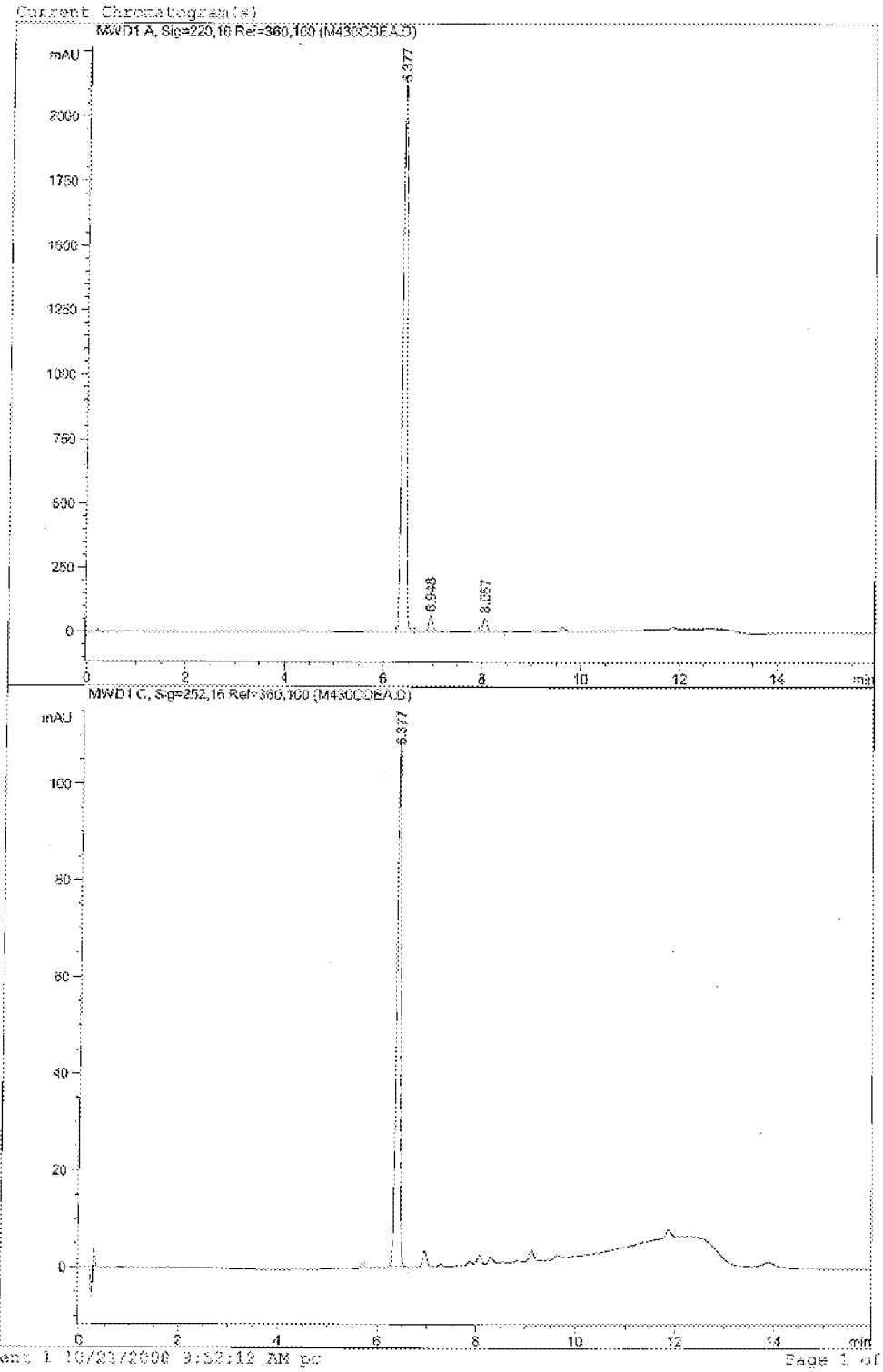
Print of window 38: Current Chromatogram(s)



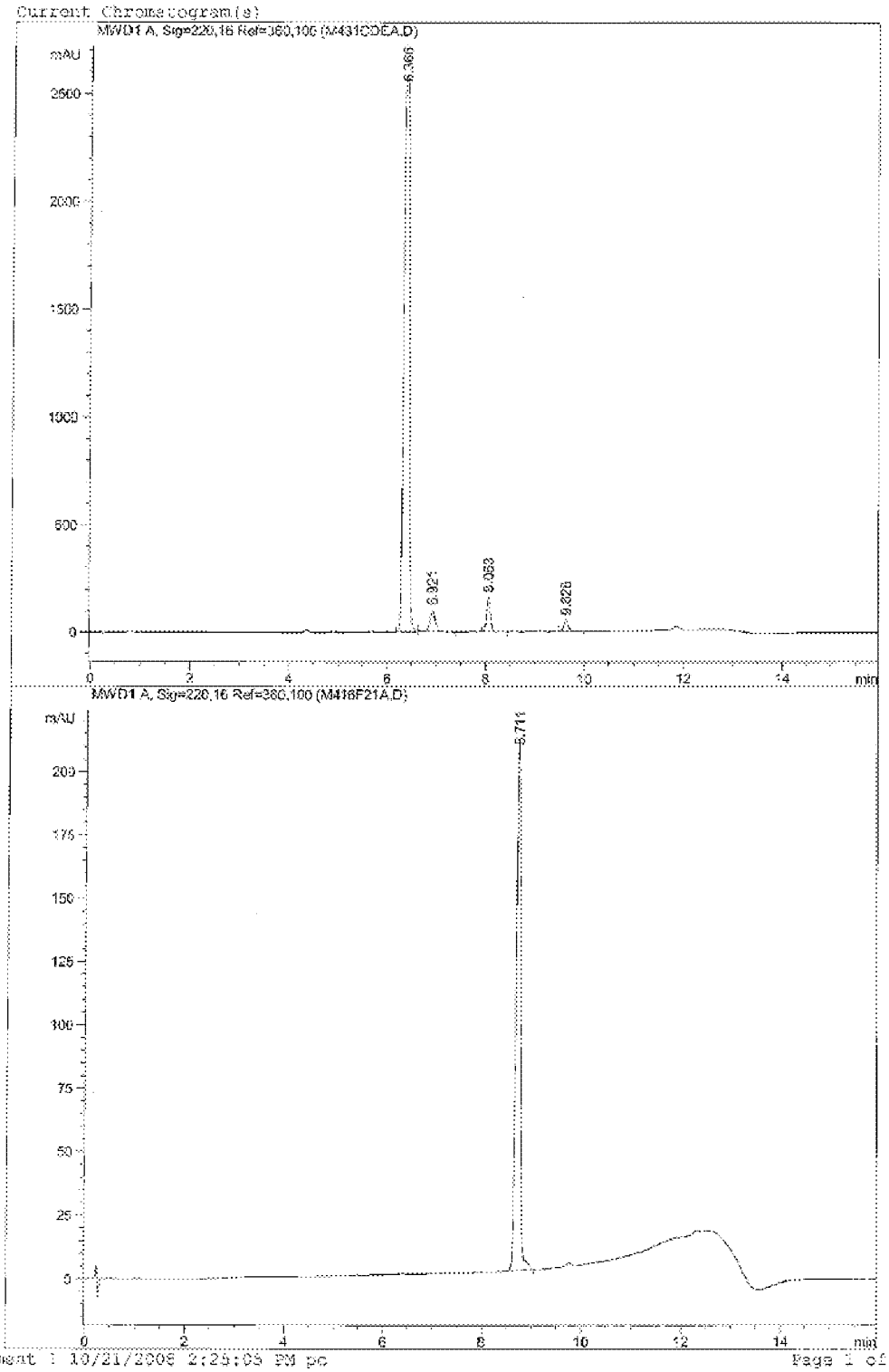
Print of window 38: Current Chromatogram(s)



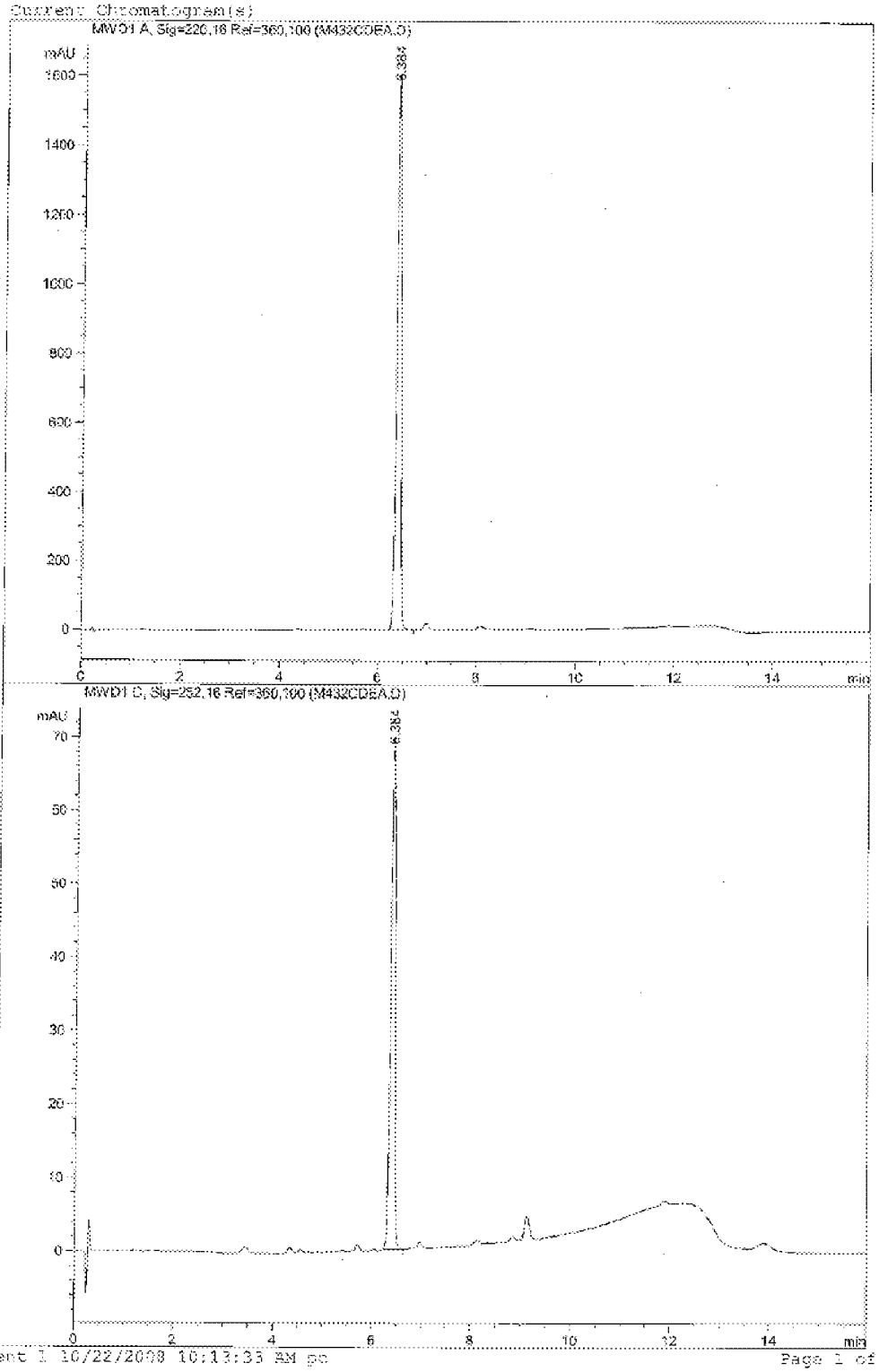
Print of window 38: Current Chromatogram(s)



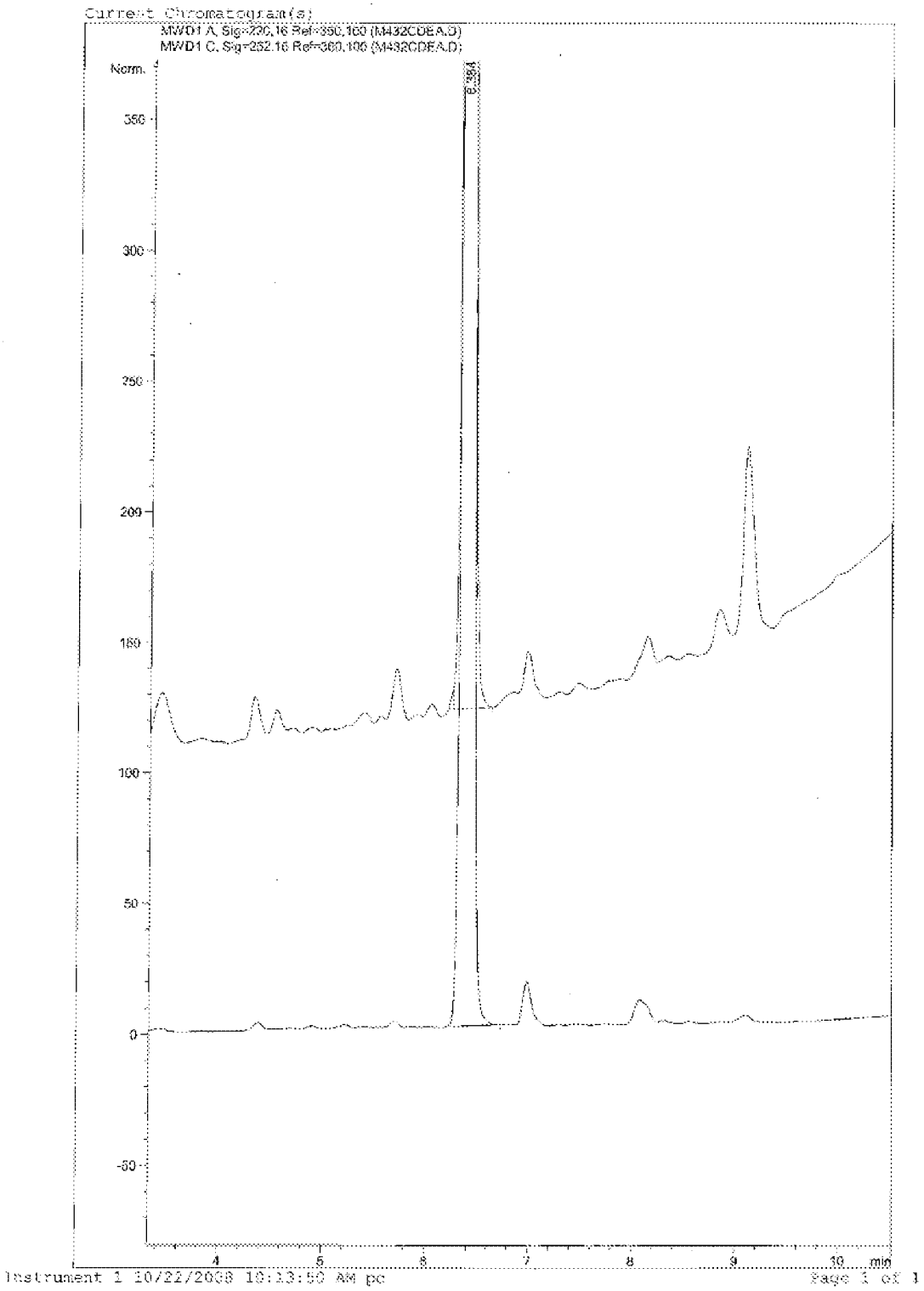
Print of Window 38: Current Chromatogram(s)



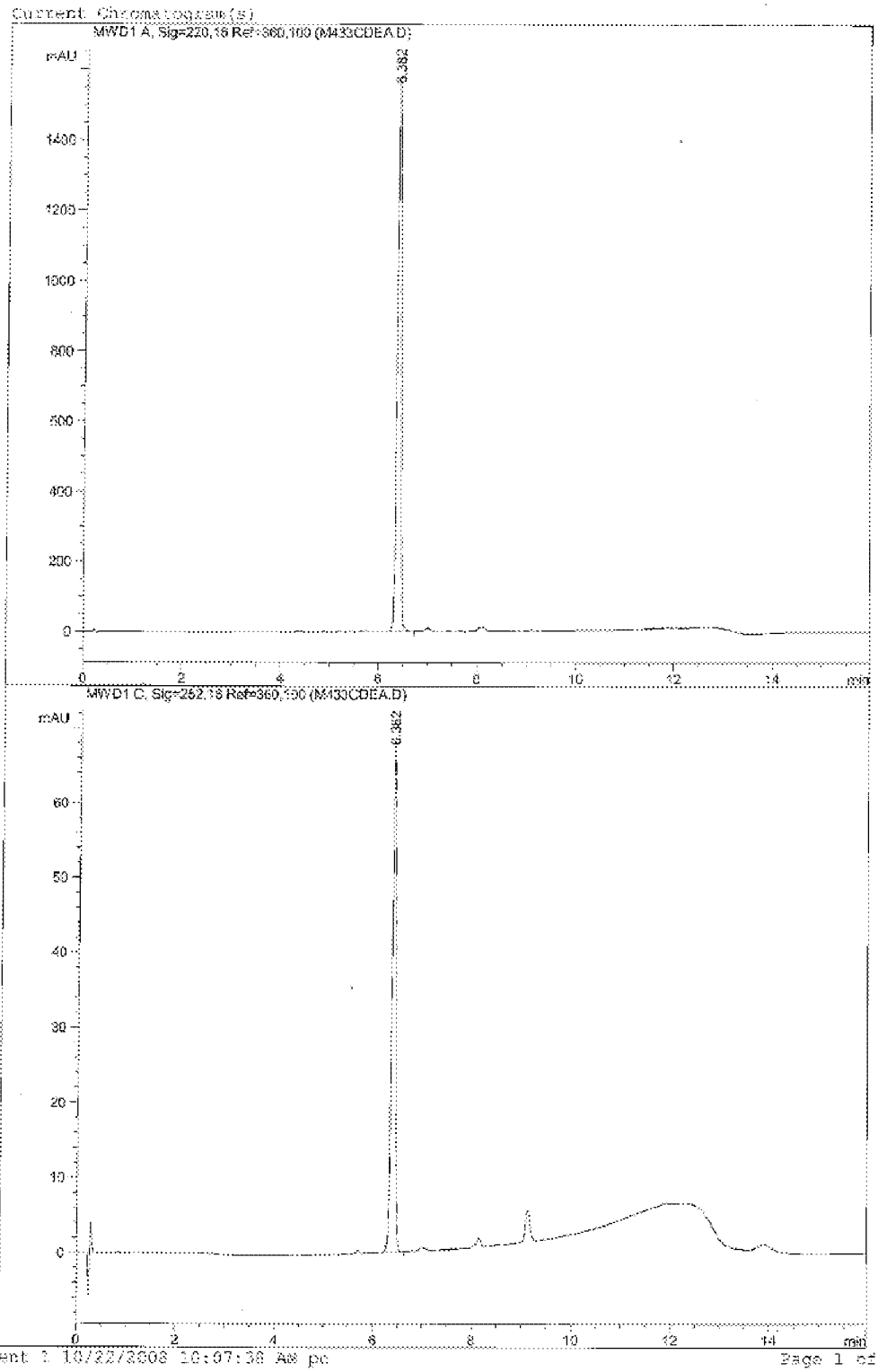
Print of Window 38: Current Chromatogram(s)



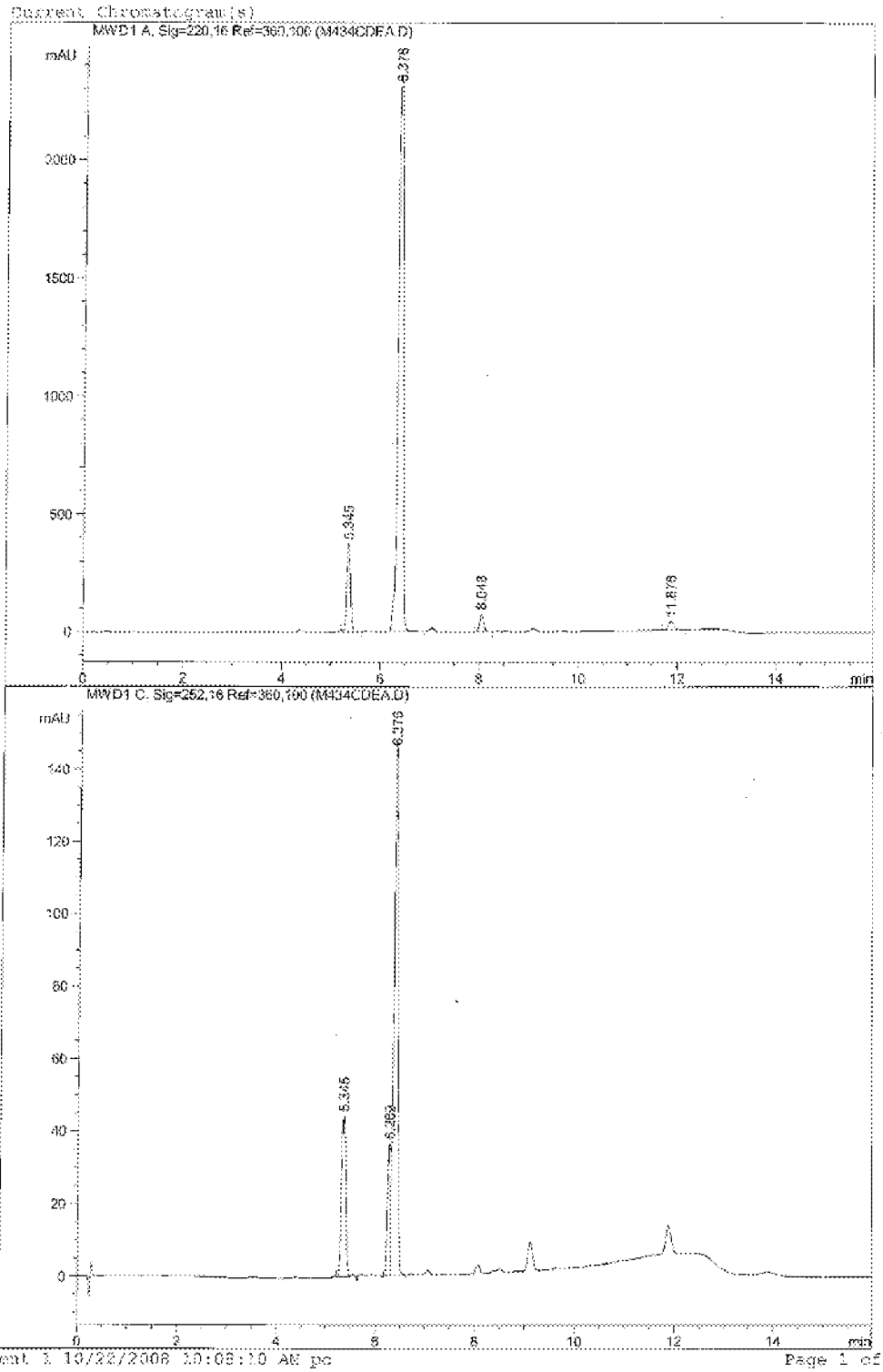
Print of window 38: Current Chromatogram(s)



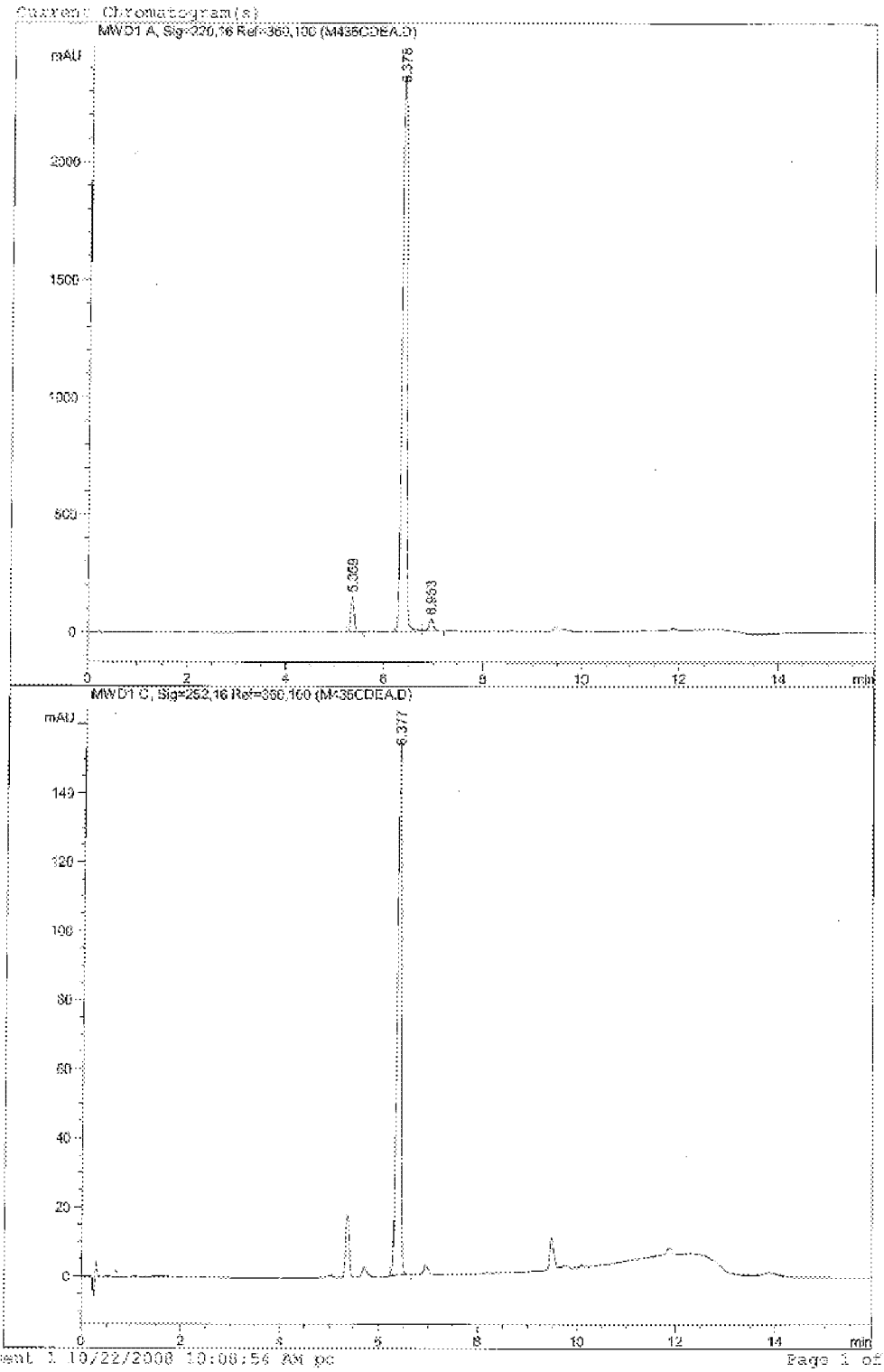
Print of window 38: Current Chromatogram(s)



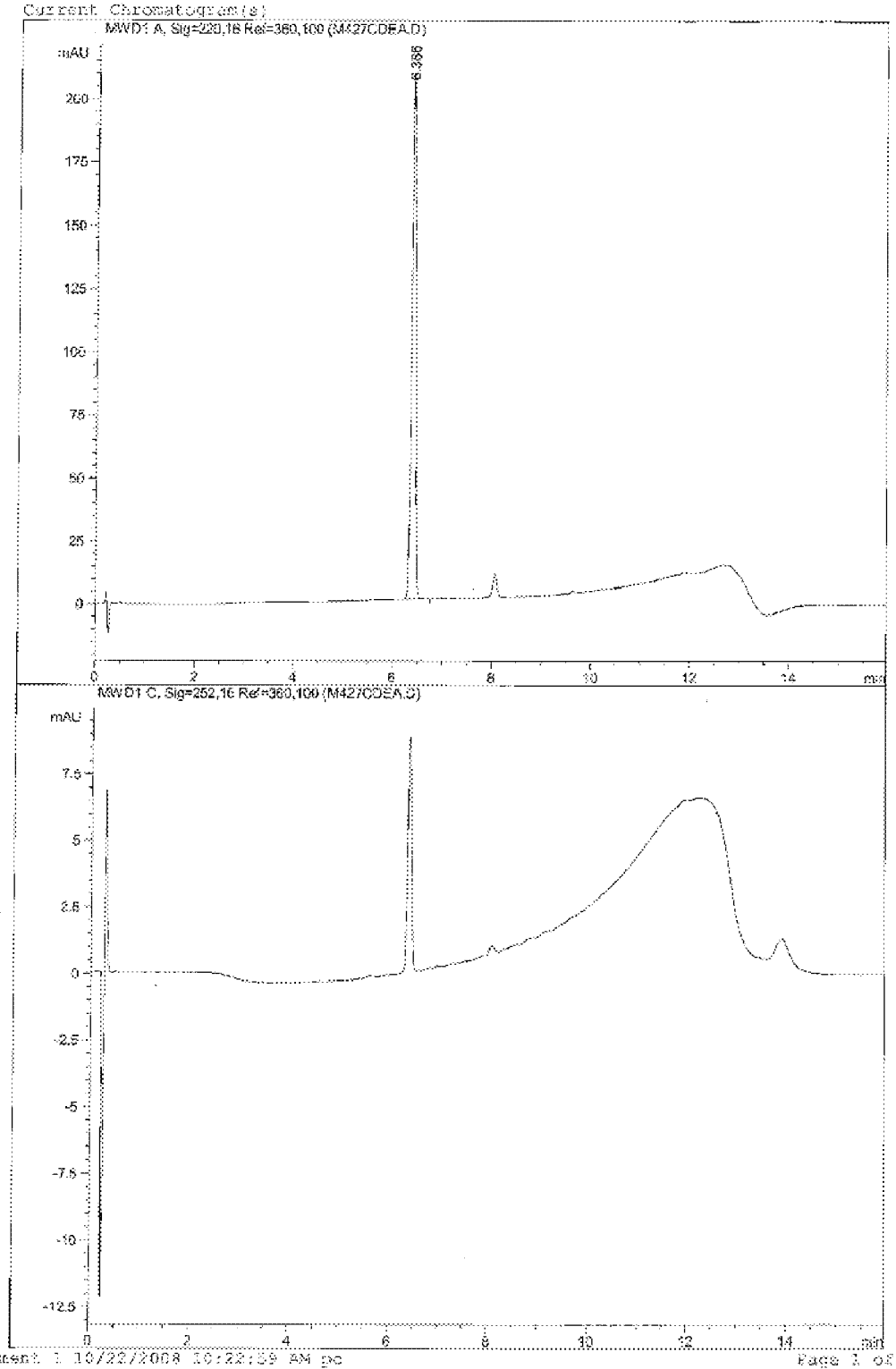
Print of window 38: Current Chromatogram(s)



Print of window 38: Current Chromatogram(s)



Print of Window 38: Current Chromatogram(s)



Print of Window 38: Current Chromatogram(s)